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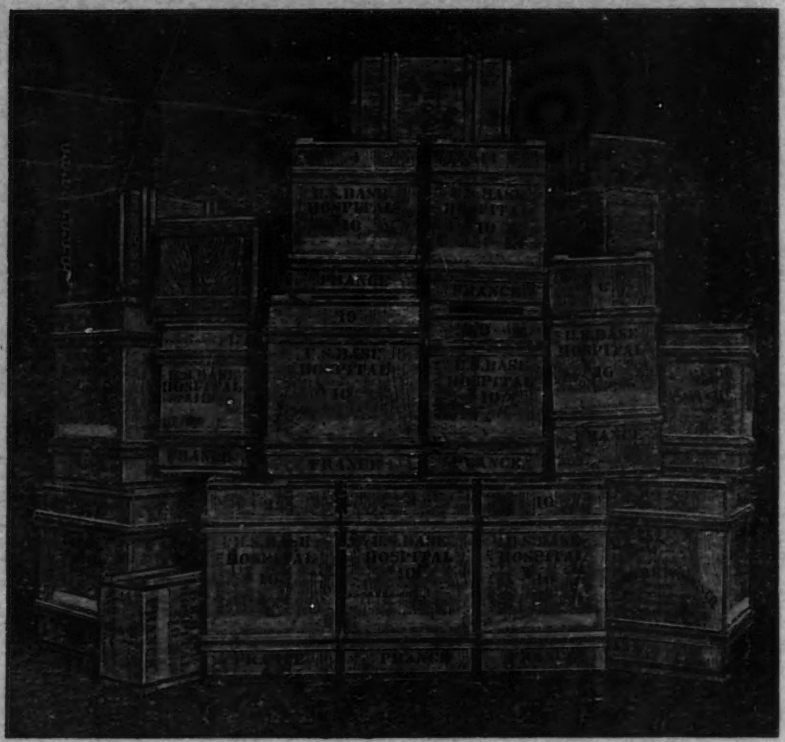

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August 6th, 1917

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WEST WASHINGTON SQUARE PHILADELPHIA, U. S. A.

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GASTRIC RESPONSE TO FOODS

I. The Determination and Significance of Intragastric Conductance[1]

OLAF BERGEIM

From the Laboratory of Physiological Chemistry, Jefferson Medical College, Philadelphia

Received for publication September 29, 1917

In connection with the series of studies being carried out in this laboratory on the response of the human stomach to various stimuli, it appeared that the determination of the electrolytic conductance of the gastric contents would be of interest in supplementing or, possibly, in part replacing other methods of study. A retention stomach tube carrying an electrolytic cell was therefore devised and an attempt made to determine the significance of variations in intragastric conductance as determined with the aid of this instrument.

The specific conductance of any solution depends only upon the number of ions present and the mobility of those ions being thus more specific than the cryoscopic index which is influenced also by the molecules of undissociated substances. Strong acids and bases or the salts of strong acids and bases are very completely dissociated in solution in the concentrations commonly met with in physiological fluids. Weak acids and bases and their salts are but slightly dissociated and neutral organic substances not salts do not ionize.

It follows that the conductance of most biological fluids is dependent upon their content of salts of strong acids and bases, especially sodium

[1]This is the first of a series of researches made possible by a grant from Mrs. M. H. Henderson.

and potassium chlorides. This is particularly true because of the relative abundance of chlorides of sodium and potassium in body fluids. Thus the blood and bile of most mammals are high in chlorides and possess considerably higher conductances than milk and saliva which are lower in chlorides.

When we study the gastric juice we at once note that while the ash of this fluid is very low as compared with blood, bile, pancreatic juice, etc., its conductance is much higher than that of these fluids. Thus while the specific conductance of bile at 18°C. is 130×10^{-4} reciprocal ohms (1), that of blood serum (2) about 110×10^{-4}, and that of saliva about 50×10^{-4}, the conductance of pure gastric juice (3) is over 400×10^{-4}. The conductance of pure gastric juice is thus due very largely to the hydrochloric acid which it contains. The total chlorine of gastric juice is not notably higher than in blood, bile or pancreatic juice but the gastric secretion conducts a current much more readily than either of the other fluids because of the great mobility of the hydrogen ion. Thus the ionic speed at 18°C. of the sodium ion is 43.5, of the potassium ion 64.6, of the chlorine ion 65.5, while that of the hydrogen ion is 318. A tenth normal solution of hydrochloric acid at 18°C. has a specific conductance of 351×10^{-4} as compared with 92.5 for sodium chloride and 111.9 for potassium chloride.

From these considerations it appeared that the determination of intragastric conductance might be used in many cases as an index of the concentration of hydrochloric acid in the stomach. A determination of this character would possess the advantage over titration methods of obtaining the desired data at as frequent intervals as desired, without any disturbance or removal of gastric contents. Titration methods for free hydrochloric acid in the presence of protein are especially unsatisfactory because of the further dissociation of the protein salt during the course of analysis. Phosphates from the saliva or other sources produce a similar effect while the coloring matter of certain foods, especially fruits, may make titration almost impossible. These difficulties are avoided by the electrometric method. The values obtained with its aid will be more accurate indexes of the hydrogen ion concentration (4) the higher the concentration of hydrochloric acid and the lower that of other electrolytes.

APPARATUS AND METHOD

The cell used in this work is illustrated in figure 1. It consists essentially of a hard rubber tip of the size used in the Rehfuss stomach tube

(about 1 cm. in diameter), slotted however in only one direction and made in four pieces for convenience in wiring. Into this tip are fitted opposite to each other the two platinum electrodes, about 10.0 × 6.0 mm. in size. All edges are of course rounded off smoothly so as to cause no irritation in swallowing. The leads consist of double or triple copper wire no. 36 about 3 feet long or longer if it is desired to pass the tube into the intestine. The conductance of the wire is appreciable and must be balanced in the other arm of the bridge. The use of heavier wire decreases the flexibility of the tube and it is desirable to maintain this. The electrodes must of course be properly platinized by the electrolysis of platinic chloride solution and must be kept clean. The cell constant is readily determined by means of $\frac{N}{10}$ potassium chloride solution whose specific conductance at 18°C. is 0.01119.

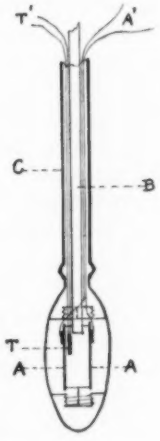

Fig. 1. Conductance cell for intragastric work. Diagrammatic cross section showing platinum electrodes *A*, *A*, with leads *A'*; thermocouple *T* with leads, *T'*; tube for aspiration *B*; and outer protecting tube *C*.

The tip with tubes attached is swallowed until aspiration shows it to be resting in the stomach. The conductance is determined in the ordinary manner using a good Wheatstone bridge, buzzer and telephone receiver. More expensive types of apparatus are not essential for intragastric work. Two or three dry cells will furnish current. A switch convenient to the bridge is desirable so that the circuit may be kept closed as much as possible. The buzzer should be enclosed in a soundproof box and placed at some distance from the bridge.

The thermocouple used is a base metal couple (copper and iron-constantan). Fine wire (0.1 mm.) is used for reasons previously mentioned and also that the current through the cell may be interfered with as little as possible.

The couple must be coated with paraffin and gastric juice must not be allowed to come into contact with any of the connecting wires. The couple is placed in circuit with a potentiometer indicator and high sensitivity mirror galvanometer.[2] A similar arrangement has been used by Stengel

[2] The tips used were made for us by Chas. Lentz & Sons, Philadelphia, and all other apparatus for conductance and temperature measurements by the Leeds & Northup Company of Philadelphia.

and Hopkins (5) in the study of intragastric temperature. By this means it is possible to observe at any moment the temperature of the stomach contents by merely adjusting the indicator until the galvanometer stands at zero and then reading off the temperature directly upon the indicator scale.

The fact that conductance increases about 2 per cent for each degree rise of temperature makes accurate observations of the latter necessary. The stomach is a fairly good thermostat if given time enough to adjust itself, but after the ingestion of cold foods or liquids of any kind, a half hour or more may be required to reach body temperature. By the aid of the thermocouple it is possible to study the conductance in this early period after the ingestion of food with a fair degree of accuracy by applying a temperature correction. The tip may also be used simply to replace dip electrodes for determination of conductances of small amounts of fluids in test tubes.

In studying the significance of intragastric conductance as determined with this apparatus the gastric contents obtained by aspiration at fifteen minute intervals were analyzed (6) and values obtained compared with conductances determined at the same intervals. Total acidity was titrated using phenolphthalein as an indicator, and free acid in most cases using Toepfer's reagent although in a few cases the Sahli iodine method was employed. Pepsin was determined by a modified Mett method. Trypsin was determined by Spencer's method (7) slightly modified. The presence or absence of bile, food residues, etc., was also noted.

EXPERIMENTAL AND DISCUSSION

A series of tests was first made using water and sugar solutions. These were practically non-conducting so that changes in conductance after introduction into the stomach would be expected, unless other diluting fluids were involved, to be due mainly to the hydrochloric acid of the gastric juice secreted in response to the stimulus. The four experiments charted in figures 2, 3 and 4 show that under these conditions the conductances of the gastric contents ran very closely parallel to the acidities (total acidity and free hydrochloric acid) and were mainly dependent upon the free hydrochloric acid present.

Conductances for convenience are plotted in whole numbers, a value of 250 signifying a specific conductance of 250×10^{-4}. All values for conductance were obtained at body temperature (37°) or corrected for deviations from this temperature. In the charts the curves for

conductance and titration values for free hydrochloric acid nearly overlap because the conductance of $\frac{N}{10}$ hydrochloric acid solution at 37°, expressed as above indicated, gives values very close to five times the titration values expressed in cubic centimeters of $\frac{N}{10}$ alkali.

These experiments show that ordinarily after the introduction of a 5 per cent sugar solution the concentration lowering is brought about

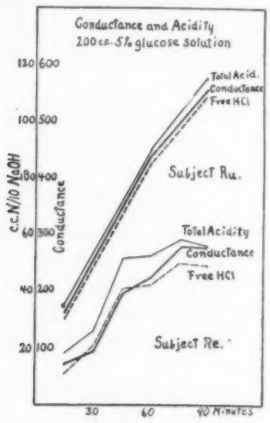

Fig. 2

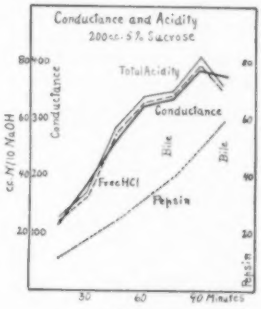

Fig. 3

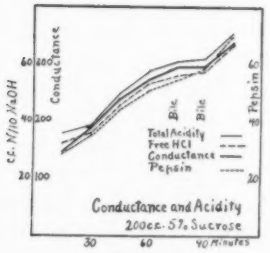

Fig. 4

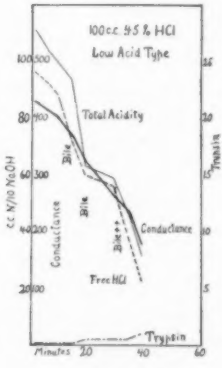

Fig. 5

by the secretion of gastric juice of normal hydrochloric acid content, and the conductance throughout the intragastric phase studied is almost entirely dependent upon the free hydrochloric acid of this secretion. A rather unusual degree of parallelism of pepsin with conductance and acidity is noted in figures 3 and 4, particularly the latter.

The effect of the introduction of 100 cc. of 0.45 per cent hydrochloric acid solution into the stomach of an individual of low acid type was studied. The results are given in figure 5. Here again we note a parallelism of conductance with free acidity, both values falling off rapidly. That the fall is due mainly to regurgitation of pancreatic juice and bile was shown by the color and general appearance of the later samples. The high concentration of hydrochloric acid has however destroyed most of the trypsin. Note the relative rise of the conductance as compared with free acid when the latter falls off toward the end of digestion.

The effect of "combined" acidity upon conductance is well shown after the administration of a protein food such as bread (see fig. 6). The values for conductance closely parallel those for free acid. They lie a little lower, however, indicating that, as would be expected, titration values for free hydrochloric acid are too high in these circumstances. The regurgitation of bile reduces both acidity and conductance but not in direct proportion. The conductance rises relatively, due to the chlorides of the bile. The "combined" acid is shown to have relatively little influence on conductance. The rise in pepsin with fall in acidity should also be noted.

The experiment charted in figure 7 illustrates the same facts as the preceding one, the parallelism of free hydrochloric acid with conductance, the high values obtained for the former by titration and the relative rise of conductance toward the end of digestion when regurgitation begins. It will be noted also that while the free acidity starts at little above zero, the conductance is already 100, so that obviously care is necessary in ascribing conductances at so low a level to hydrochloric acid.

The preceding experiments were carried out on normal individuals and indicate the usual response under the conditions of study. Figures 8 and 9 illustrate experiments on an individual with near-achylia. They illustrate clearly the point just mentioned that great care is necessary in interpreting changes in conductance when the values lie below 150.

In the experiment illustrated in figure 8, 500 cc. of distilled water were given. The conductance rises slowly at first and then very rapidly to 150, although the free hydrochloric and even the total acidity remain nearly negligible throughout. The reason for this is clearly brought out by a study of the tryptic values. Marked regurgitation takes place so that the final sample is nearly pure pancreatic juice.

In the second experiment (fig. 9) 250 cc. of 10 per cent sucrose solution were given. The result was similar to that of the preceding experiment except that evacuation was somewhat delayed by the sugar present. In both of these cases then there was noted a parallelism

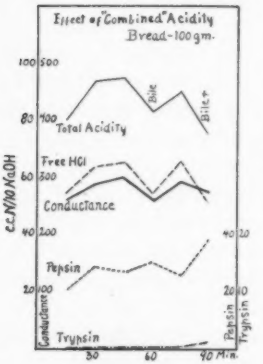

Fig. 6

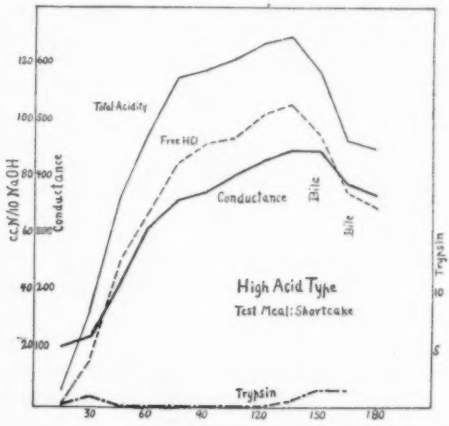

Fig. 7

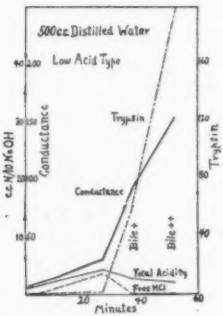

Fig. 8

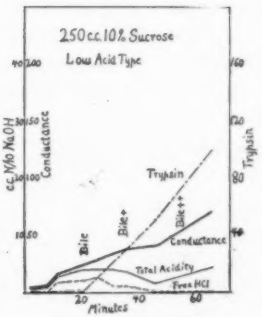

Fig. 9

of conductance with trypsin values (that is, with the concentration of pancreatic juice), gastric secretion being entirely secondary in its influence. Such findings could of course only be expected in achylias.

More typical of normal gastric digestion are the curves given in figure 10. Here the total acidity begins high due to organic acid

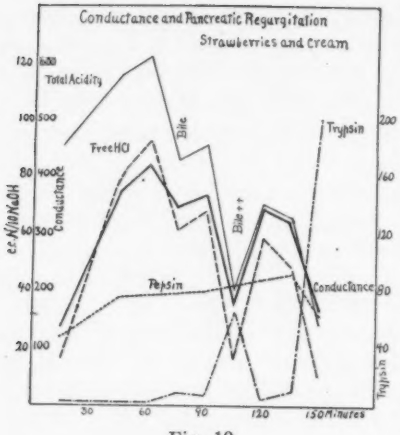

Fig. 10

which has but little influence on conductance. After about an hour regurgitation of pancreatic juice and bile begins as indicated by the tryptic values, also by the color of samples withdrawn. Both acidity and conductance fall sharply, rise due to secondary gastric secretion and again fall due to further regurgitation. The last specimen is very high in trypsin and is nearly pure pancreatic juice. Here again may be noted the rise of conductance relative to free acidity, so that the final sample has hardly any free acid, but has a conductance of 165.

Figure 11 shows somewhat similar conditions. Here, however, the tryptic index remains very low and the intense green color which the samples developed on standing showed the regurgitated fluid to be almost entirely bile. The reduction of acidity is more gradual than in the preceding case and the conductance falls off less rapidly. Pepsin, as in the preceding case, remains nearly constant in spite of the dilution of the gastric juice by regurgitation. The reason for this is not entirely clear. Certain results of this type indicate that the acidity lowering may be due in part to the slightly alkaline duodenal and pyloric secretions which contain pepsin. It has not been shown however that the pepsin contents of these fluids are sufficiently high to explain some of the results obtained. In the absence of regurgitation the pep-

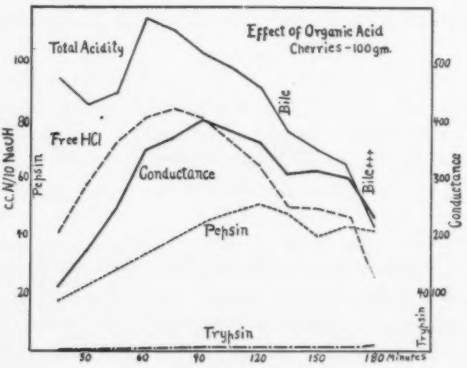

Fig. 11

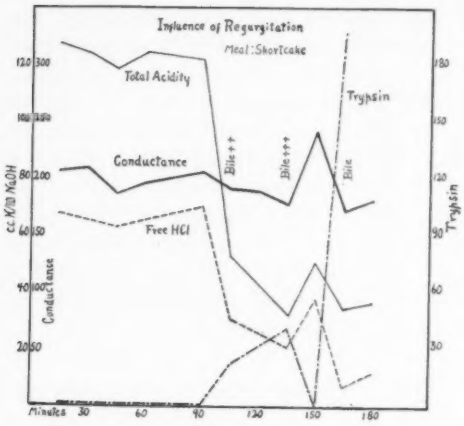

Fig. 12

sin rises with the acid, showing a certain parallelism. Regurgitaion brings about a neutralization of acid, while pepsin is not destroyed. Hence the latter will be expected to rise relatively.

The organic acid of the fruit ingested in this case causes titration values for acidity to lie considerably above the true curve for free hydrochloric acid which would more nearly follow the curve for conductance. This illustrates the value of conductance determinations as a check on free acid where organic acid is present.

The experiment illustrated in figure 12 is unusual in certain respects. Here we have very marked regurgitation of pancreatic juice and extreme changes in acidity with but little influence on conductance. In this case during the first hour and a half the acidity (even a part of that titrating as free hydrochloric acid) is due to organic and "combined" acids which are low in conductance. The conductance is so low in fact that the values obtained are but slightly above those for pure pancreatic juice and bile. Hence admixture with these secretions produces only slight lowering of conductance.

In figure 13 we have represented a case of almost perfect regulation of gastric acidity by means of regurgitation. The acidity remains nearly constant for a period of two and a half hours. As long as the acidity thus remains constant the rise in conductance parallels that of trypsin. When the acidities

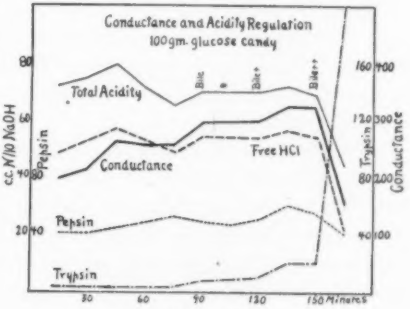

Fig. 13

become markedly reduced, however, the relation of trypsin to conductance becomes inverse because of the lower conductance of pancreatic juice.

In this case no water was taken and the solution entering the stomach was salivary in character and hence relatively high in phosphate. This accounts for the marked difference between free and total acid which we regularly find in such cases. The conductance of the alkali phosphates being low, the conductance curve more nearly follows that for free hydrochloric acid. Several samples of saliva tested by us have shown a conductance of 50–60 $\times$ 10^{-4} at 37°. This is appreciably lower than values given in the literature although filtered saliva may have been employed in earlier determinations.

SUMMARY AND CONCLUSIONS

A retention stomach tube in the form of an electrolytic cell has been devised which makes possible the determination of intragastric conductances at any desired interval of time without disturbance or removal of gastric contents. It may also be used in place of dip electrodes. The tip contains a thermocouple which makes possible intragastric temperature determinations and corrections, and an aspiration tube by means of which samples of gastric contents may, if desired, be collected for analysis.

By means of this apparatus intragastric conductance variations were studied in connection with determinations of total acidity, free hydrochloric acid, pepsin and trypsin. The conductance of gastric juice is mainly due to the free hydrochloric acid which it contains and the same is usually true of the gastric contents.

After the introduction of water or solutions (as sugar solutions) of very low conductance, the curve for conductance very closely follows the curve for free and total acid. This indicates that the equalization of osmotic concentration is brought about primarily by secretion of normal gastric juice.

After the ingestion of food containing protein the conductance curve usually lies below that for free hydrochloric acid as determined by titration because the latter values are high due to gradual dissociation of the protein salt. In the presence of weak organic acid as after fruit ingestion or of phosphate as where much saliva is swallowed, the conductance falls below titration values and is a better measure of free hydrochloric acid.

Aside from the swallowing of saliva, the conductance of which is low, intragastric conductance is, after the first hour or so of digestion, almost always considerably modified by the regurgitation of pancreatic juice or bile or both and possibly to a lesser extent by pyloric and duodenal secretions. The conductance of pancreatic juice and bile being usually very low as compared with that of the gastric contents at maximum acidity, regurgitation tends to markedly lower intragastric conductance as well as acidity. Conductance, however, rises relative to free hydrochloric acid on account of the higher salt content of these regurgitated secretions. After the ingestion of mineral acid, neutralization is brought about in the same manner as during digestion.

In achylias where intragastric digestion is mainly pancreatic in character the conductance was found to parallel the concentration of pancreatic juice as measured by the tryptic index.

Studies of intragastric digestion by this method as well as of the influence of salt solutions in the stomach and upper intestine, are being continued.

The author is indebted to Dr. P. B. Hawk, Dr. C. A. Smith and Mr. R. J. Miller for the privilege of using certain of these cases and data. He desires also to thank Messrs. H. S. Sargent and E. L. Small for assistance in enzyme determinations.

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AN AIR-TIGHT PLEURA CANNULA

S. J. MELTZER

From the Department of Physiology and Pharmacology of The Rockefeller Institute

Received for publication October 1, 1917

In the beginning of the nineties I devised an air-tight pleura cannula. The late Prof. Hugo Kronecker who in 1893 saw the efficient working of the cannula in my private laboratory asked me to give him a brief description of it; this sketch he published later in two journals (1). At the tenth annual meeting of the American Physiological Society (2) I showed the cannula in connection with another demonstration, but published no description of the cannula itself.

About fifteen years ago, I made an essential change in the construction of the cannula. It is the latter construction which we have been using in our laboratory since 1904. The cannula was often mentioned in papers which emanated from our department, but we never published a description of its construction. In connection with the following paper of Dr. A. L. Meyer, in whose investigation the cannulas played an essential part, I decided to publish the following brief description of our pleura cannula in its present form.

Figure 1 presents the later type of pleura cannula when all its parts are connected; the rubber gasket and two leather washers are here omitted.

Figure 2 shows the four parts composing the pleura cannula.

Part 1. When the larger branches are put together, the entire parts presents a T-shaped hollow cylinder terminating in two flat plates (feet). The vertical cylinder carries on about three-fourths of its length a spiral ridge (thread). The feet which taper toward the bevcled end are flat on the lower surface and slightly rounded on the upper side. Both halves of the cylinder hinge at the heel. When they are separated to 90 degrees the entire part assumes again a T-shape in which both feet form one branch that stands perpendicular to the horizontal lines formed by the halves of the cylinder.

Part 2 is a rosette shaped plate with an opening in the middle which is slightly larger than the diameter of the cylinder of part 1. Around

the opening are arranged about twenty or more sectors of a flat S shape. The sectors are elastic and when the plate is pressed down it acts like a spring and adapts itself to the elevations and grooves of the thoracic wall.

Part 3. This is simply an external (female) screw adapted to the thread of the cylinder in part 1.

Part 4 presents a cylinder the lower part of which is again an external (female) screw of the same bore as the previously mentioned screw (part 3). At the end of the screw the cylinder carries a stopcock and

Fig. 1

Fig. 1. The newer type of the pleura cannula; the rubber gasket and two leather washers are here omitted.

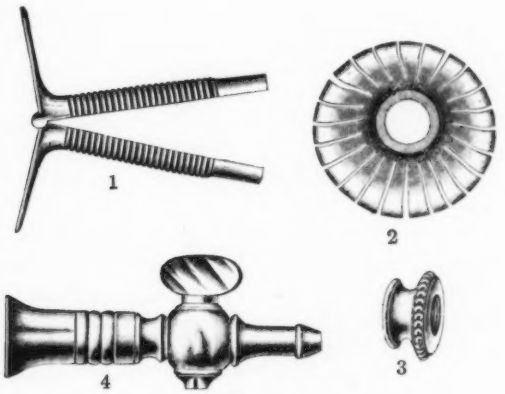

Fig. 2

Fig. 2. The four essential parts composing the cannula. The description is given in the text.

terminates with a conical thickening for the making of an air-tight connection with rubber tubing.

For the introduction and fastening of the cannula the procedure is to be as follows: In the fourth intercostal space, at about the junction between the anterior and middle part of the ribs, a small incision is made with a knife through the skin and the muscles and the pleura is broken with a blunt instrument of a small diameter. The feet of part 1 are now put together and pushed through the small opening into the pleural cavity. Now each half of the cylinder is raised to 90 de-

grees until they form an externally protruding tube while the separated feet are now resting on the opposing ribs and at right angles to them. Over the cylinder a round gasket of soft rubber, with an opening in the middle, is now pushed down to the skin and is followed by the rosette shaped plate (part 2); a leather washer is pushed down to the plate and then the external small screw (part 3) is screwed down very tightly so as to keep the cylinder in place and adapt the sectors of the rosette shaped plate to the uneven parts of the thoracic wall. While this is done the cylinder should be grasped firmly so that the position of the feet in relation to the ribs should remain unchanged. Now follows another leather washer, upon which part 4 is tightly screwed down. The collapsed lung is now being distended and the stopcock so turned that no air can enter again into the pleural cavity.

In dogs the distention of the lung can be done either by blowing through a tracheotomy tube or still better, through an intratracheal tube introduced through the mouth and larynx; in the latter case it is well to exert briefly a slight pressure just above the larynx. For rabbits (and cats) there is a simpler method of distention. The trachea should be compressed while the abdomen and the liver are pressed upwards; the air of the uncollapsed lung has no other place for escape than to enter into the collapsed lung.

The protruding end of the pleura cannula is now to be connected by means of tubing with a Marey tambour. The tubing has to have a T-tube bearing a pinch cock. While the stopcock of the cannula is still closed the pinch cock is opened and an atmospheric line is drawn on a revolving smoked drum. After closing the pinch cock and opening the stopcock of the pleura cannula, the respiration is marking its undulations upon the revolving drum. If everything is done properly, the tops of the expiratory elevations remain below the atmospheric line —an evidence of the existence of the negative pressure in the pleural cavity. It is essential that the rosette plate be tightly screwed down. When this is the case, no change in the relations of the respiratory movements to the atmospheric line is taking place except, of course, under special conditions (pressure upon the abdomen, strong active expirations, stimulations of the vagus, inferior or superior laryngeal nerves, etc.). It may happen occasionally that by over-distending the lung the pleural opening into the cannula may become closed up by a slip of the lung. This can be remedied by compressing the thorax in the dorso-abdominal direction. But it is altogether not necessary to overdistend the lung; the presence of a very small amount of air in the

pleural cavity will not interfere with most of the conditions which we wish to study. I wish to mention the further fact that in pushing in the feet of the cylinder it may happen that no sufficient opening in the pleura is made and that the feet may even remain outside of the parietal pleura. It is therefore advisable that the feet, before permitting them to separate, should be pressed well down into the pleural cavity, and furthermore, before screwing on part 4 a probe should be pushed into the cylinder in order to obtain evidence that the pleural cavity is freely connected with the cannula.[1]

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[1] The cannula may be obtained from Georg Tieman & Company, 107 Park Row, New York City.

A NEW METHOD OF OBTAINING THE RESPIRATORY GASES IN ANIMALS, AND SOME OF THE RESULTS

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INTRODUCTION

The purpose of this paper is to present a new method of obtaining a sample of air from the lungs of animals. The method, in essence, consists in the introduction of air under pressure into both pleural spaces, the lungs being thereby completely compressed and their contents forced into a rubber bag attached to a tracheal cannula. In this manner all the air is collected with the exception of a small quantity which remains in the uncollapsible portion of the system, namely the bronchi, trachea and cannula (dead space). The bag therefore contains a mixture of *supplementary* and *residual* air.

The procedures hitherto employed fall roughly within two groups. During the latter third of the nineteenth century samples of pulmonary air were obtained after either the entire air within the lungs or only a portion of the air had been brought into equilibrium with the gases of the venous blood. This is essentially the principle of the methods adopted by Pflüger and his school. To mention only a few names of this school, Wolffberg (1) and Nussbaum (2), experimenting on dogs, used Pflüger's lung catheter which enabled them to occlude a portion of the pulmonary air until equilibrium had been established. The percentages of carbon dioxid which they found were distinctly lower than those obtained later by Loewy and von Schrötter (3). These investigators applied the same principle in human beings and succeeded in giving the technique greater refinement with the aid of bronchoscopy. Plesch, (4) dispensing with the use of the catheter but still adhering to the same principle, had his subjects rebreathe air from a rubber bag until the mixture of gases within the system had become homogeneous and had reached the same tension as the carbon dioxid and

oxygen of the venous blood. This method in modified form has enjoyed fairly extensive use in clinical investigation (5).

In all of these procedures the air within the lungs is modified and its analysis, consequently, does not represent the composition of the air normally present in the lungs at any single moment. In the group next to be considered samples of pulmonary air are obtained without first subjecting it to conditions which materially modify its composition. While these methods are pretty generally understood, I shall nevertheless mention them in some detail for purposes of comparison with my own method.

In 1905 Haldane and Priestly (6) introduced a method of obtaining a sample of alveolar air in human beings. The subject takes a comfortable position and waits until his respirations have become regular. At the end of a normal inspiration he expires suddenly and very deeply through a rubber tube 1 inch in diameter and about 4 feet long, provided with a mouthpiece, and instantly closes the tube by applying his tongue to the mouthpiece. From the portion of the tube nearest the mouth a sample is then withdrawn. In a second experiment, a sample is taken at the close of deep expiration, following a normal expiration. The mean result of the percentages thus obtained represents the mean composition of the alveolar air. In the case of J. S. H. the average percentage of alveolar carbon dioxid was 5.62, while the highest percentage was 5.87 and the lowest 5.40. In the case of J. G. P. the average percentage was 6.28; the highest 6.85 and the lowest 5.98.

Boycott and Haldane (7) made five determinations during a period of one and a half hours at a room temperature of 18.4° to 19.0°C. The actual percentages of carbon dioxid were 5.42, 5.66, 5.62, 5.34, 5.52. Fitzgerald and Haldane (8) studied the variations in carbon dioxid percentages of the alveolar air during two successive nights and days. There were twenty-five determinations. They state that the figures of the last twenty-four hours were probably influenced by exhaustion. If then we take the fourteen percentages from 5.45 p.m. to 6.15 p.m. which are means of two or three determinations, we obtain an average of 4.93; the percentages showing maximum deviation from the mean were 4.73 and 5.21; those showing a minimum deviation, 4.91 and 4.96. In another experiment carried out from 8 a.m. to 7.30 p.m., there were seven determinations with an average of 4.81. In this case the percentages showing the widest deviation from the mean were 4.62 and 4.95, while those with the smallest deviations were 4.80 and 4.86.

The differences between individual determinations carried out according to the method of Haldane and Priestley are easily understood when one remembers that the depth of successive respirations nearly always varies in most persons. Not only is its use difficult in certain pathological cases owing to the deep expiratory effort required, but experience shows that even in normal individuals its successful appli-

cation depends upon an intelligent and willing coöperation on the part of the subject.

Lindhard (9) has described what may be considered a modification of the Haldane-Priestley method. A sample of air is obtained at the close of several expirations. The respirations take place through a valved apparatus and an air-tight mask covering nose and mouth. A narrow lead tube ending immediately below the expiratory valve and opposite the mouth leads to a sampling tube filled with mercury. The top of the receiver is turned at the end of an expiration. A small sample is taken. This is repeated until the fractional samples total 50 cc. The experience of Boothby and Peabody (10) has shown that the essential point in Lindhard's method is the exact time at which the samples are collected. Furthermore it was found difficult to obtain accurate samples with a tidal air of but 300 cc. They proceeded to modify the method to the extent of not only omitting the valve and placing the lead tube as far back in the mouth as was consistent with comfort but of requesting the subject to expire 600 to 800 cc. upon a given signal, whereupon the tap was turned and 3 to 5 cc. were withdrawn. This was repeated until a sufficiently large sample had been obtained. Successive samples in one subject gave partial pressures of 38.8; 40.9; 39.1; 38.8 mm. Hg. The results are in agreement with those found by the Haldane-Priestley method.

The only effort to obtain a sample of the unmodified pulmonary air in animals by a method conforming in principle to my method is that of Scott (11). He experimented on cats and analyzed the last portion of the air obtained by compression of the thorax and abdomen.

Scott finds that great variations occur in the same animal during the course of an experiment. Ether and urethane were used as the anesthetic agents. During the period between 10.23 and 1.30, seven analyses were made. The body temperature fell 3.5°C. Two samples taken at an interval of seventeen minutes showed the percentages of carbon dioxid to be 4.68 and 4.23; two other samples twenty minutes apart contained 4.25 and 4.04 per cent of carbon dioxid. Two successive samples taken at an interval of one hour showed no difference. From 1.44 to 5.30 in the same experiment eight determinations were made. The average percentage of carbon dioxid was 4.39, while the percentages showing maximum deviation were 4.21 and 4.53; those showing minimum deviation were 4.34 and 4.44. During the first six determinations of this period the body temperature rose about 3°C; during the last two it fell.

In the Haldane-Priestley method and its modifications, as well as in the method used by Scott the sample for analysis consists of only a small part of the air within the lungs, either the last portion of a forced expiration, or an expiration somewhat deeper than the normal tidal air of the subject, or else the last part of the air obtained by forcible compression of the thorax and abdomen. In each case a considerable volume of air remains in the lungs. When manual compression of the

thorax is practiced in young cats one may possibly greatly diminish the residual air remaining in the lungs, but I believe it is quite unlikely that one could thereby force out all the residual air.

METHOD

Referring to the foregoing remark I wish to state that, while it is not the purpose of the method presently to be set forth to analyze especially the residual air, nevertheless the residual air is in large measure recovered by this method and together with the entire supplementary air constitutes the sample for analysis.

In the present series the experiments were made on dogs that had been without food for about twenty-four hours. Chloretone, dissolved in olive oil, was used as the anesthetic. It was administered intraperitoneally, in the dose of 0.24 to 0.30 gram per kilogram of body weight Fifteen to thirty minutes later tracheotomy done in the usual manner was followed by the insertion and firm fixation of a T-shaped glass cannula. Next a special cannula,[1] devised by Meltzer (12) and used in this laboratory for some years, was placed in each pleural cavity, in the fourth right and fifth left intercostal spaces, sufficiently near the lateral line to avoid the thick sternal and pectoral muscles. Once the pleural cannulas had been placed it became necessary to redistend the lungs and reëstablish the intrathoracic negative pressure. This was readily accomplished by the introduction into the tracheal cannula of air under a pressure of 40 mm. Hg. maintained for fifteen seconds. This pressure was found to be most effective if the abdomen was coincidently compressed. It is also well in order to insure free exit of air from the pleural spaces during inflation to compress alternately the anterior and posterior regions of the thorax. The stopcocks of the cannulas were closed before the inflation was discontinued. As a matter of fact the maximum negative pressures are rather to be avoided owing to the frequency with which the lungs block the internal openings of the cannulas. Such an accident would obviously interfere with the kymographic record and be apt to give an erroneous impression both of the amount of negative pressure and the character of the respirations. For this reason I have thus far used negative pressure somewhat below the maximum. How successfully one might omit the kymographic record and work with the maximum negative pressure, has not been established.

[1] The use of this cannula for the present purpose is due to the suggestion of Doctor Meltzer.

It is always important to test the pleural spaces for air-tightness. It should be possible to maintain a negative pressure within the thorax of 70 mm. H_2O for an indefinite period. If any change occurs it ought to be in the direction of an increase rather than a decrease because of the gradual absorption of gases from the pleural cavities. The pleural cannulas were therefore connected by means of a Y-piece and suitable rubber tubing with a Marey tambour and a water manometer. A record of the negative pressure and respiration could then be made upon smoked paper.

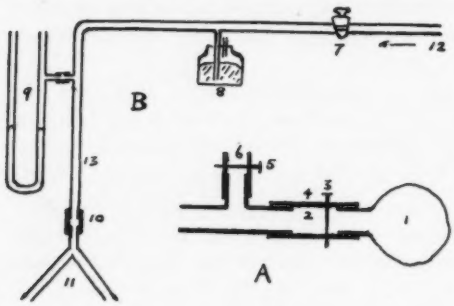

Fig. 1. Diagram *A* shows rubber bag attached to a T-shaped glass cannula. Bag may be closed by means of screw-clamp *3*. Rubber tubing *6* may be closed with a pair of hemostatic forceps *5*. *B* represents the pressure connections. Air entering at *12* passes into pleural spaces through Y-piece *11* attached to pleural cannulas, when stop-cock *7* is open. Tubing *13* can be disconnected at *10* and attached to the tracheal cannula for inflation of the lungs.

Now as to the procedure immediately preceding the sample: A small sized rubber bag of approximately 100 cc. having been attached to the tracheal cannula as indicated in figure 1, the pleural cannulas were connected by means of a Y-piece with a source of air pressure. A mercury valve introduced at *8* allowed the escape of air in excess of the desired pressure (40 mm. Hg). The next steps followed in quick succession. While compressing the rubber tube *2* of the bag with the forefinger and thumb of the left hand, the screw-clamp *3* and stopcocks of the cannulas were opened; closure of the cannula at *6* immediately at the end of the next expiration was followed by the opening of the pressure valve *7* and release of forefinger and thumb at *2*. The entrance of air into the pleural sacs compressed the lungs and forced their contents into the rubber bag. The filling of the bag was completed in about the time it requires to make a forced expiration. The remaining part of the procedure consisted in the opening of the tracheal cannula at *6*, the disconnection of the air pressure and the reëstablishment of the negative pressure. While apparently complicated, with a little practice the steps involved need not consume one minute.

In analyzing the air in the bag I followed the technique described by Haldane in connection with his smaller apparatus (13) It is of great importance that the rubber bag should not be too large. An ordinary nitrous oxid bag is much too large for the volume of air obtained from a dog of 6 to 10 kgm. It was found that the analysis of two samples taken from a large bag differed widely. The size of the bag must be such as to permit rapid and complete diffusion. On the other hand, the bag must not be so small as to offer resistance and prevent the entrance of the full amount of air.

SOME RESULTS

It was important to determine at the outset whether analyses of samples collected in this manner would yield uniform results in one and the same experiment. In the present series of experiments (table 1) no precautions were taken to maintain the body temperature of the animal. Heat was applied in certain experiments of another series but the results will be reserved for a subsequent paper. Furthermore only the carbon dioxid was studied; no analyses were made of the oxygen.

In all of the experiments uniformity in the percentages was exhibited either immediately or after a variable preliminary period. In table 2 the experiments appear in condensed form. The second column of this table shows the time that elapsed between the first and last samples of each experiment, while the third column indicates the time during which the percentages remained uniform.

In the course of three and a half hours seven samples were obtained and analyzed in the first experiment. It is evident that constancy was present from the beginning. The average percentage of the period was 5.37; the percentages showing maximum deviation from the average were 5.23 and 5.51.

The first three samples of the second experiment showed fluctuation in the percentages, whereupon the carbon dioxid became uniform and continued so for a period of over three hours, during which eight samples were obtained. The percentages showing maximum deviation from the average of 6.03 were 5.85 and 6.24.

In the third experiment the first four analyses again presented marked differences. Uniformity in the carbon dioxid was observed however for a period of two hours beginning at 2.32 p.m. During this time eight samples were taken. The average figure for the period was 5.82 while those exhibiting maximum divergence from the mean were 5.65 and 6.01.

TABLE 1

DATE AND NUMBER OF EXPERIMENT	TIME OF SAMPLE	INTERVAL BETWEEN SAMPLES	BODY TEMPERATURE	CARBON DIOXID	DATE AND NUMBER OF EXPERIMENT	TIME OF SAMPLE	INTERVAL BETWEEN SAMPLES	BODY TEMPERATURE	CARBON DIOXID
				per cent					*per cent*
Feb. 26	12.03		37.23	5.31	Mar. 8	12.34		36.22	4.87
1	12.26	23	36.92	5.35	4	12.59	25	35.98	4.80
	1.35	50	36.56	5.48		1.22	23	35.70	4.83
	2.01	25	36.35	5.51		2.22	60	35.00	4.76
	2.42	40	36.13	5.35		2.48	26	34.80	4.79
	3.09	27	36.05	5.23		3.06	18	34.70	4.95
	3.37	24	35.95	5.34		3.30	24	34.55	4.64
						3.48	18	34.42	4.87
Mar. 1	11.32		36.39	5.66					
2	11.52	20	35.95	6.05	Mar. 12	2.16		31.50	5.64
	12.07	15	35.70	5.75	5	2.45	29	30.90	5.53
						3.07	22	30.50	5.59
	12.20	13	35.45	6.15		3.35	25	30.05	5.63
	12.37	17	35.20	5.94		3.54	19	29.60	5.64
	12.53	16	34.98	6.09					
	1.52	60	34.25	6.10	Apr. 12	1.53			5.43
	2.10	18	34.05	5.95	6	2.15	22		5.66
	2.30	20	33.85	5.85		2.28	13		5.41
	3.06	36	33.60	5.95		2.41	13		5.49
	3.45	40	33.40	6.24		2.56	15		5.35
						3.26	30	34.15	5.20
Mar. 7	12.16		36.50	6.15					
3	1.00	44	36.30	5.58	Apr. 25	11.47			6.34
	1.12	12	35.85	5.78	7	12.08	21		6.26
	1.23	11	35.88	6.08		12.18	10		5.90
	2.32	50	35.60	5.69		12.30	12		5.56
	2.53	20		5.65		1.16	46		5.48
	2.56	3	35.25	6.01		1.28	12	33.95	5.53
	3.20	24	35.02	5.94		1.41	13		5.45
	3.42	22	34.85	5.88		1.53	12	33.80	5.42
	3.59	17	34.72	5.70		2.12	19		5.52
	4.18	19	34.53	5.87		2.25	13	33.65	
	4.32	14	34.45	5.79					

The carbon dioxid in the fourth experiment was constant for a period of over three hours. Eight analyses were made in this time. The percentages with the maximum deviation from the mean of 4.81 were 4.64 and 4.95. One of the percentages was comparatively low owing to a marked increase in the rate and depth of the respirations.

TABLE 2

DATE OF EXPERIMENT	DURATION OF EXPERIMENT	PERIOD OF UNIFORMITY	CHANGE IN RECTAL TEMPERATURE DURING EXPERIMENT	AVERAGE PERCENTAGE CARBON DIOXID	MAXIMUM AND MINIMUM PERCENTAGES	NUMBER OF UNIFORM DETERMINATION
1917			°C.			
February 26	12.03–3.37	12.03–3.37	−1.3	5.37	5.23; 5.51	7
March 1	11.32–3.45	12.20–3.45	−3.0	6.03	5.85; 6.24	8
March 7	12.16–4.32	2.32–4.32	−2.0	5.82	5.65; 6.01	8
March 8	12.34–3.48	12.34–3.48	−2.0	4.81	4.64; 4.95	8
March 12	2.16–3.54	2.16–3.54	−2.0	5.61	5.53; 5.64	5
April 12	1.53–3.26	1.53–3.26		5.42	5.20; 5.66	6
April 25	11.47–2.25	12.30–2.25		5.49	5.42; 5.56	

Uniformity in the percentages of carbon dioxid was immediate in the fifth experiment and continued for more than one and a half hours. During this period five samples were analyzed. The average was 5.61 while 5.53 and 5.64 were the figures showing the greatest deviation from the mean.

Again, in the sixth experiment the carbon dioxid exhibited uniform behavior with a period of preliminary fluctuation. The period of observation covered one and a half hours and during this time six determinations were made with an average of 5.42, the highest and lowest percentages being respectively 5.66 and 5.20. The respirations in this animal were quite irregular in depth.

In the last experiment three determinations in the first half hour fluctuated; these, however, were followed by a period of uniformity lasting two hours during which six samples were obtained averaging 5.49, with a minimum percentage of 5.42 and a maximum of 5.56.

There was no persistent upward or downward tendency in the percentages of any one experiment. In nearly all cases the last percentage of the period of uniformity was identical with the first percentage of the period. This is rather interesting in view of the steady fall in the body temperature. A fall in body temperature seemed rather to occasion a fall in the percentages of carbon dioxid in Scott's (14) experiments.

I am unable to state definitely the cause of the preliminary fluctuations sometimes observed. It is significant however that in all those experiments in which analyses were not begun until 12.00 m. or later this phenomenon did not appear, except in the experiment of March 7 in which the breathing in the beginning was of the periodic type.

Chloretone was always given between 9.30 and 10.00. It is possible that the volatile nature of this substance may have interfered with the analysis of the air.[3]

The method is clearly capable of a variety of modifications and ought to be useful in the solution of a number of problems bearing on the physiology of the respiration. The percentages in the above experiments are not absolute values and hence the results of one animal must not be compared with the results obtained in another animal. This is partly to be accounted for by the fact that the negative pressures were not the same in the different dogs; in some more air was allowed to remain in the pleural sacs than in others. Neverthless it is evident that the percentages of carbon dioxid in samples obtained by this method may exhibit uniformity for periods as long as three and one-half hours and that the variations are no greater than one obtains by the use of other methods.

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[3] Among other factors that may play a rôle is the possible alteration in the volume of the dead space.

THE VISCOSITY OF LYMPH

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As the method which has been made use of by Burton-Opitz (1) in determining the viscosity of different body-fluids has been described in an earlier paper, it need only be mentioned at this time that the experiments now under discussion purpose to ascertain the factors required to calculate the coefficient of the viscosity of lymph. The apparatus is arranged in such a way that it is possible to measure how large a quantity of this fluid escapes through a capillary tube of known length and diameter in a given period of time and under a certain pressure. The coefficient derived from these factors is then compared with the coefficient for distilled water at 37°C. which, in accordance with Poiseuille (2) equals the value 4700.

The present experiments were performed upon dogs which had received a moderate amount of fatty meat about four hours previously. Light ether narcosis was employed. The lymph was gathered from the central orifice of the thoracic duct. It became necessary at times to hasten its flow by exerting gentle pressure upon the abdominal wall, because as lymph clots very rapidly, its passage through the viscosimeter could not be permitted to consume a longer time than about one minute. From 2 to 3 cc. of lymph were used for each determination. The specific gravity was ascertained with the help of small pycnometers possessing a capacity of about 4 cc.

The results of these determinations are compiled in table 1. It will be seen from this that the viscosity of lymph varies considerably. The coefficients here recorded lie between the figures 2406 and 3029 and present the average value 2682.5. If this coefficient is compared with the coefficient for distilled water of 37°C., it will be found that lymph possesses a viscosity which is 1.7 greater than that of water. It must be emphasized, however, that these determinations have been made during the absorption of fat and that, therefore, these values are some-

what above those obtainable with perfectly clear lymph. The nearest approach to the watery type of lymph was yielded by dog 5, this experiment having been performed almost six hours after the ingestion of food. The relatively high numerical value of this coefficient (3029)

TABLE 1

EXPERIMENT NUMBER	SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT OF VISCOSITY	DIFFERENCE	AVERAGE VALUE
		mgm.	*seconds*	*mm. Hg.*			
1	1.0170	1.9613	72.07	103.2	2661.3	40.7	2681.6
	1.0170	1.2592	47.70	98.6	2702.0		
2	1.0168	1.8990	74.80	91.5	2800.5	17.5	2818.0
	1.0168	1.6494	49.60	118.4	2835.6		
3	1.0141	2.4285	68.98	138.9	2565.1	119.4	2624.8
	1.0141	1.6201	50.10	121.6	2684.5		
4	1.0140	2.1238	59.08	132.7	2754.0	43.6	2732.2
	1.0140	1.9332	58.08	124.3	2710.4		
5	1.0119	2.8210	64.10	145.4	3069.9	80.5	3029.6
	1.0119	2.7177	66.05	139.6	2989.4		
6	1.0230	2.2998	59.50	149.8	2588.7	72.3	2624.8
	1.0230	2.4687	66.20	140.6	2661.0		
7	1.0194	2.5081	64.13	133.3	2976.8	35.9	2994.7
	1.0194	2.0758	53.20	131.4	3012.7		
8	1.0171	1.7019	64.20	110.0	2432.1	50.9	2406.6
	1.0171	1.2840	57.16	95.2	2381.2		
9	1.0119	1.8746	61.93	127.4	2410.1	26.1	2423.1
	1.0119	1.4588	52.63	115.4	2436.2		
10	1.0215	1.5828	49.22	130.7	2472.1	36.2	2490.2
	1.0215	1.6215	51.60	125.9	2508.3		

indicates that this lymph possesses a viscosity only 1.5 times greater than that of distilled water of 37°C.

In a similar way, it may be gathered that increasing absorption heightens the viscous resistance of the lymph. Thus, the coefficient 2406 obtained by experiment 8 shows that this lymph is almost 2.0

times more viscous than distilled water of 37°C. In this connection, brief reference might be made to the fact that the viscosity of blood is almost 5 times greater than that of distilled water (3) while that of ox bile (4) is only 1.8 and that of saliva only 1.4 times greater (5).

The specific gravity follows a course parallel to that of the viscosity. The values recorded above vary between 1.0119 and 1.0230. The average value is 1.0165. The coagulation-time which was determined by the method of inversion of a small test tube filled with a small quantity of fresh lymph, varied between one and seven minutes. The average time was two minutes and a half. It is evident, therefore, that

TABLE 2

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT	AVERAGE VALUE	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*			
1	Normal	1.0230	2.2998	59.50	149.8	2588.7	2624.8	524.1
		1.0230	2.4687	66.20	140.6	2661.0		
	During stimulation	1.0285	1.3729	54.12	119.4	2120.5	2100.7	
		1.0285	1.2661	52.61	115.4	2081.0		
2	Normal	1.0194	2.5081	64.13	133.3	2976.8	2994.7	421.6
		1.0194	2.0758	53.20	131.4	3012.7		
	During stimulation	1.0228	1.5643	49.81	120.5	2695.2	2573.1	
		1.0228	1.4805	51.63	117.4	2451.0		

viscosity experiments upon lymph necessitate the same precautionary measures as those made upon the circulating blood. The time required for the completion of each test should not be longer than one minute.

Table 2 is intended to illustrate the changes which the lymph of the thoracic duct undergoes in consequence of the stimulation of the greater splanchnic nerve. The experiments with which we are concerned at this time, are those designated in table 1 as 6 and 7. In both cases the left greater splanchnic nerve was used, shielded electrodes having been applied to it through a small opening in the abdominal wall. Having collected a sufficient quantity of lymph for the determination of the normal viscosity and specific gravity, the two subsequent collections were made while the aforesaid nerve was being stimulated with a tetanic current of medium strength.

TABLE 3

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT	AVERAGE VALUE	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*			
1	Normal	1.0141	2.4285	68.98	138.9	2565.1	2624.8	+133.9
		1.0141	1.6201	50.10	121.6	2684.5		
	After injection: 100 cc.	1.0175	1.3934	48.50	115.0	2520.0	2490.9	
		1.0175	1.4847	53.10	114.6	2461.0		
2	Normal	1.0140	2.1238	59.08	132.7	2754.0	2732.2	−38.9
		1.0140	1.9332	58.08	124.3	2710.4		
	After injection: 200 cc.	1.0145	2.2431	68.02	120.0	2780.2	2772.1	
		1.0145	1.7745	58.40	116.4	2764.1		
3	Normal	1.0119	2.8210	64.10	145.4	3069.9	3029.6	−48.9
		1.0119	2.7177	66.05	139.6	2989.4		
	After injection: 300 cc.	1.0110	2.2705	61.10	121.6	3102.4	3078.5	
		1.0110	1.9056	52.60	120.4	3054.7		

TABLE 4

EXPERIMENT NUMBER		SPECIFIC GRAVITY	QUANTITY	TIME	PRESSURE	COEFFICIENT	AVERAGE VALUE	DIFFERENCE
			mgm.	*seconds*	*mm. Hg.*			
1	Normal	1.0119	1.8746	61.93	127.4	2410.1	2423.1	−637.0
		1.0119	1.4588	52.63	115.4	2436.2		
	After hemorrhage and injection	1.0102	1.8196	53.35	114.6	3024.0	3060.1	
		1.0102	1.8756	54.61	112.7	3096.2		
2	Normal	1.0215	1.5828	49.22	130.7	2472.1	2490.2	−325.4
		1.0215	1.6215	51.60	125.9	2508.3		
	After hemorrhage and injection	1.0180	1.8855	61.70	110.6	2785.7	2815.6	
		1.0180	1.4697	50.80	102.5	2845.6		

The stimulations resulted in each case in reductions in the discharge of lymph, each reduction being ushered in by a brief period of increased flow. The lymph collected during these periods presented a much greater viscous resistance than the normal lymph and showed, moreover, a much greater specific gravity. Thus, a comparison of the coefficients of experiment 6 will show that this procedure has increased the viscosity by 524 points. In other words, the normal relationship of 1:1.7 has been changed in this case to a relationship of 1:2.2. This alteration is also indicated by the specific gravity, because the normal value of 1.0230 has given way to the value 1.0285. While, therefore, the stimulation of the splanchnic nerve leads to a lessened production of lymph, the lymph transferred at this time into the duct is not only more concentrated but also more viscous.

Table 3 illustrates the changes in the viscosity of the lymph resulting in consequence of the introduction into the venous circulation of varying quantities of normal saline solution. The injections were made shortly after the completion of the determinations of the normal viscosity. From 100 to 300 cc. of saline were injected at a time and about fifteen minutes were allowed to elapse before the viscosity was again tested.

The results show that large doses of normal saline solution tend to lessen the viscosity in a slight measure. Smaller doses, on the other hand, may increase it, presumably on account of the fact that the more favorable dynamic conditions subsequent to the injection tend to augment the absorption and the transfer of the lymph to central channels. As is illustrated by table 4, a very rapid reduction of the viscosity results, however, if the injections of saline solution are preceded by a moderate hemorrhage. Under this condition, very naturally, the blood and lymph pressures must suffer, allowing a more prompt "thinning" of the latter to take place. In the experiments here cited 200 cc. of blood were displaced by an equal quantity of saline solution. The tests were made fifteen minutes after the injection.

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STUDIES ON ANIMAL DIASTASES

II. The Effect of the Administration of Various Substances on the Blood Diastase of Rabbits

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In 1846 Magendie (1) demonstrated the existence of starch-splitting enzymes in drawn blood and sought to demonstrate their existence in the living blood by the injection of boiled starch. Claude Bernard, Schiff, Tiegel, Pavy, Tieffenbach, Böhm and Hoffman found sugar in the urine after the intravenous injection of starch or glycogen. Bial (2) was one of the first investigators who studied the question of blood and lymph diastase with improved methods but Röhman (3) is credited with having first proved the existence of diastase in the living body. He injected glycogen into a lymph vessel of the dog's leg and found an excess of sugar in the lymph drawn from a thoracic duct fistula. Fischer and Niebel (4) proved conclusively the presence of diastatic enzymes in blood and lymph.

Claude Bernard (5) discovered that a puncture of the floor of the fourth ventricle was followed by a transitory form of diabetes. Later Cavazzani (6) found that stimulation of the coeliac plexus caused an increased sugar production in the liver and Levene (7) obtained the same results by stimulation of the vagus. Carlson and Luckhardt (8) concluded that if the liver was the chief source of blood and lymph diastase, the increased glycolytic activity of the liver caused by nervous stimulation might involve an increased output of the liver diastase into the blood and lymph. They found this to be the case.

In cases of definite anatomical lesions the diastatic activity increased in both serum and urine. Schlesinger (9) observed an increase of blood diastase after ligating the duct of Wirsung. Clerc and Loeper (10) also found an increase in blood diastase after ligating the pancreatic duct in rabbits. Gould and Carlson (11), working with dogs,

reported that the ligation of the pancreatic ducts is followed by a great increase in the diastatic power of the serum within twenty-four hours and assumed this to be due to absorbed amylopsin. King (12) states that there is an increased diastatic activity in both serum and urine after ligation of the pancreatic ducts. Wohlgemuth and Noguchi (13) have reported that in cases of abdominal trauma where the pancreas was injured, there is a quantitative increase of amylase in the urine; this was confirmed by King (12).

Bainbridge and Beddard (14) and Carlson and Luckhardt (8) recorded that the removal of the pancreas from cats did not greatly affect the concentration of blood diastase. Schlesinger (9) reports, on the other hand, that pancreatectomy leads to complete disappearance of amylase from the blood. Otten and Galloway (15) found after complete removal of the pancreas in dogs that the blood diastase sinks rapidly, then rises somewhat, remaining at a constant level but never returning to its normal height. King (12) also claims a reduction but not a complete disappearance. Later Milne and Peters (16) obtained a decided increase of blood diastase after complete pancreatectomy.

Van de Erve (17) found that the removal of the kidney has no appreciable effect on the diastase in serum.

King (12) reported that after profound depression, for example, in parathyroid tetany, there is a high uniform content of amylase in the urine.

Many investigators have found that in some types of experimental hyperglycemia and glycosuria there is an increase of diastase at the same time in the blood and the urine.

In cases of human diabetes, Loewi, Moeckel and Rost, and more recently Myers and Killian (18) have reported an increased diastase in the blood. In the impairment of renal function, Loeper, Ficai, Hirata, Stocks, Myers and Killian have found an increase of diastatic activity in the blood and a decrease in the urine. In nephritis, Wohlgemuth, Corbett and King noted a decrease in the amylase content as compared with that of normal urine.

The experiments detailed in the present paper were undertaken to determine the influence of the administration of certain drugs and human saliva on the diastatic activity in the blood and urine.

METHODS

A roughly quantitative method for the estimation of diastase was devised by Wohlgemuth in 1908 (19). He based his method on the hydrolysis of starch into erythro-dextrin by amylase. Recently Myers and Killian (20) have suggested a more accurate method for the determination of the diastatic activity in blood and urine, based upon the hydrolysis of starch to glucose. This method was employed in the present investigations. Since the diastatic activity of rabbit blood is more than double that of human blood, one cc. of blood was employed for the test instead of the 2 cc. used by Myers and Killian for human blood. The calculations are for the one cc. For the determination of sugar in the blood and urine, the Myers and Bailey (21) modification of the Lewis-Benedict method was used. While this method does not admit of the accurate determination of the absolute amount of sugar in the urine, comparative results are very easily obtained and it is with these that we have to do in this investigation.

In all the following tables the percentage of blood sugar is higher than in normal rabbits, this being probably due to the feeding of bread, milk and barley before the experiments. The rabbits used in these experiments were injected with several types of drugs at different periods but no single drug was used twice on the same rabbit. A sufficient period of time was allowed to elapse after the first injection to permit all effects of the drug to pass away before a second injection. The rabbits used were all full grown. The blood drawn from the ear vein was treated with potassium oxalate.

Intravenous injection of human saliva. The technic of the collection of saliva. To collect saliva, the mouth was washed several times with distilled water and the saliva was then collected in sterilized centrifuge tubes. The flow of saliva was stimulated by chewing paraffin. By centrifuging the saliva in an electric centrifuge at full speed for over one-half hour, most of the bacteria and solid material were removed and the supernatant fluid was then used for injection into the ear vein.

King (12) injected saliva intravenously into dogs and stated that more than 2.5 cc. of human saliva per kilo body weight produced signs of collapse resembling an anaphylactic shock. In our experiments we found that rabbits tolerated more than double the dose given by King.

Two rabbits weighing 1750 grams and 1900 grams, respectively, were each injected with 5 cc. saliva and showed no symptoms whatso-

ever. A second injection of 10 cc. of saliva was made and rabbit 2 died with symptoms of septicemia. Rabbit 1 showed no symptoms and was again injected with 10 cc. saliva, but without deleterious effect.

In table 1 we see that after the injection of 5 cc. saliva there is an increased diastatic activity in the blood, which ceases after forty-eight hours. This fact is in harmony with King's experiments. When 10

TABLE 1

The sugar and the diastatic activity of the blood after injection of human saliva

RABBIT 1				RABBIT 2			
Date	Blood sugar	Diastatic activity	Amount of saliva injected	Date	Blood sugar	Diastatic activity	Amount of saliva injected
1916	*per cent*			*1916*	*per cent*		
12/20	0.16			12/20	0.13		
12/21	0.13	25	5 cc. at 11.20 a.m.	12/21	0.13	31	5 cc. at 11.30 a.m.
12/22	0.15	33		12/22	0.12	36	
12/23	0.11	20		12/23	0.10	20	
12/24	0.15	24		12/24	0.14	26	
12/25	0.15	29	10 cc. at 12.00 m.	12/25	0.12	29	10 cc. at 12.00 m.
12/26	0.15	47		12/26	0.15	45	
12/27	0.15	34		12/27	0.12	40	
12/28	0.18	32		12/28	0.10	36	
12/29	0.14	36		12/29		33	
12/30	0.16	30					
12/31	0.19	35					
1/1/1917	0.18	34	10 cc. at 2.30 p.m.				
1/2	0.16	54					
1/3	0.16	30					
1/4	0.16	34					

cc. saliva are injected, the increased diastatic activity does not stop after forty-eight hours but continues for several days.

Parenteral administration of soluble starch. King (12) gave 100 cc. of 2 per cent starch solution daily to a cat and 300 cc. to a dog by stomach tube and stated that there was no appreciable change in the amylolytic activity of the urine in these animals. However, after an intravenous injection of starch into two dogs, he obtained an increase of amylase in the urine, which reached its maximum on the second day after the injection and lasted about four days.

We administered soluble starch solution several times intraperitoneally to two rabbits, with a lapse of some days between each administration. Blood was drawn daily at 9.00 a.m. and urine collected from 9.00 a.m. to 9.00 a.m. the following day, toluene being used as a preservative. The amylolytic activity of the blood increased on the

TABLE 2

The sugar and the diastatic activity of the blood after the injection of starch solution

RABBIT 3				RABBIT 4			
Date	Blood sugar	Diastatic activity	Amount of starch injected into peritoneal cavity	Date	Blood sugar	Diastatic activity	Amount of starch injected into peritoneal cavity
1917	*per cent*			*1917*	*per cent*		
1/8	0.16	32	1 gram starch in 50 cc.	1/8	0.14	32	1 gram starch in 50 cc.
1/9	0.14	36	H_2O at 2.30 p.m.	1/9	0.15	31	H_2O at 2.30 p.m.
1/10	0.17	24		1/10	0.22	25	
1/11	0.13	35		1/11	0.13	37	
1/12	0.14	32		1/12	0.13	37	
1/13	0.14	32	0.5 gram starch in 25 cc.	1/13	0.13	35	0.5 gram starch in 25 cc.
1/14	0.14	36	H_2O at 2.30 p.m.	1/13	0.16	40	H_2O at 2.30 p.m.
1/15	0.15	35		1/15	0.15	37	
1/16	0.15	39		1/16	0.14	36	
1/17	0.14	32	3 grams starch in 100	1/17	0.14	32	3 grams starch in 100
1/18	0.13	35	cc. H_2O at 3 p.m.	1/18	0.14	32	cc. H_2O at 3 p.m.
1/19	0.14	36		1/19	0.14	32	
1/20	0.13	33		1/20	0.14	38	
1/21	0.12	34		1/21	0.13	39	
1/22	0.14	36	7 grams starch in 150	1/22	0.15	39	7 grams starch in 150
1/23	0.12	36	cc. H_2O at 3.30 p.m.	1/23	0.19	33	cc. H_2O at 3.30 p.m.
1/24	0.12	37		1/24	0.11	39	
1/25	0.13	35		1/25	0.13	39	
1/26	0.16	40		1/26	0.18	42	
1/27	0.14	38		1/27	0.14	42	
1/28	0.12	38		1/28	0.16	48	
1/29	0.13	37		1/29	0.13	41	

day following the injection of starch with the exception of the first injection of rabbit 4. The dose in this case, however, was small. In the last two injections large doses were given and we found that the activity of amylase failed to increase and in some cases decreased. However, in cases where large doses were given the amylolytic activity

was elevated on successive days, especially in rabbit 4. The variation in the amount of diastase in normal rabbits is very large from day to day. It can be said with certainty, however, that the amylase content of the blood increases quantitatively after the injection of starch and also that the diastase is used to a greater extent than usual after the injection of large doses of starch. This explains the fact that following large doses of starch, there is no immediate increase of blood diastase. The urine was collected only after large doses of starch and the results

TABLE 3

The sugar and the diastatic activity of the blood before and after administration of $NaHCO_3$

RABBIT 11				RABBIT 12			
Date	Blood sugar	Diastatic activity	Remarks	Date	Blood sugar	Diastatic activity	Remarks
1917	*per cent*			*1917*	*per cent*		
2/27	0.10	32	Blood usually taken at 9 a.m.	2/27	0.09	31	Blood usually taken at 9 a.m.
2/28	0.12	30		2/27	0.09	29	
3/1	0.14	37	5 grams $NaHCO_3$ in 25 cc. H_2O at 10 a.m.	3/1	0.12	32	5 grams $NaHCO_3$ in 25 cc. H_2O at 10.30 a.m.
3/2	0.12	36		3/2	0.13	48	
3/3	0.17	35	Blood taken at 2 p.m.				Blood taken at 2.30 p.m.
3/4	0.21	35					
3/5	0.13	31					
3/6	0.14	34	10 grams $NaHCO_3$ in 50 cc. H_2O at 3.30 p.m.				
3/7	0.21	32					
3/8	0.16	34	Blood taken at 1 p.m.				
3/9	0.14	36					
3/10	0.14	32					
3/11	0.19	29					
3/12	0.15	25					

were identical with King's findings. It is certain that an increased diastase in the blood is followed by an increased elimination in the urine.

The effect of $NaHCO_3$ *administered parenterally upon the blood and urine diastase.* For this experiment two rabbits were each injected intravenously with 5 grams $NaHCO_3$ dissolved in 25 cc. H_2O. Both the blood sugar and amylase rather increased. After an interval of five days, one of the rabbits was again injected intravenously with 10 grams $NaHCO_3$ dissolved in 50 cc. H_2O and it was noted that the blood sugar and amylase increased more than usual.

King pointed out that after he fed a cat with 5 grams $NaHCO_3$, the urine was approximately neutral and the diastase content of the urine was decreased. In our case after the injection of 5 grams $NaHCO_3$, there was a marked decrease of diastase in urine which lasted two days; when 10 grams $NaHCO_3$ were injected, this marked decrease lasted for three days. This decreased activity of diastase may be due to the alkaline urine since the blood diastase remained high during this period.

Two rabbits were injected intraperitoneally with 10 grams and 5 grams $NaHCO_3$ dissolved in 50 cc. H_2O, respectively. Blood was drawn four times from these rabbits, an hour after the injection and at hourly intervals thereafter. The blood sugar and diastase increased

TABLE 4

The blood sugar and the diastatic activity of the blood in normal rabbits

RABBIT NO.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY OF THE BLOOD				
	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
37	0.14	0.14	0.14	0.13	0.14	35	35	34	35	35
39	0.15	0.14	0.16	0.15	0.15	23	24	23	21	23
40	0.16	0.15	0.16	0.16	0.16	33	32	31	31	30
41	0.17	0.16	0.14	0.15	0.17	23	23	22	22	21
Average	0.15	0.15	0.15	0.15	0.16	28	28	27	27	27

after each successive bleeding. Both rabbits died on the day of the experiment.

The normal variation of the sugar and the diastase of the blood during successive hours. The above group of rabbits was used as an aid in comparing the various types of investigations. As Table 4 shows the individual variation in blood sugar and diastase within four to five hours is very slight, although there is a wide variation between the blood sugar and diastase in different rabbits. Blood was drawn before and after the administration of the drug and the results compared.

The effect of the intraperitoneal administration of Na_2CO_3 *solution on the blood diastase.* Underhill (22) has shown that in normal rabbits the intravenous injection of Na_2CO_3 solution causes a hypoglycemia lasting for one or one and one-half hour. Underhill and McDanell (23) repeated the same experiments, but failed to obtain the above results. However, they (24) confirm the statement of Underhill that the hyperglycemia and glycosuria provoked by epinephrin are both

significantly decreased, if Na_2CO_3 is administered at a suitable period of time previous to the epinephrin introduction. We injected Na_2CO_3 into the abdominal cavity and found no appreciable change in the sugar and diastase content of the blood.

TABLE 5

The sugar and the diastatic activity of the blood before and after administration of Na_2CO_3 solution

RABBIT NO.	AMOUNT OF Na_2CO_3 SOLUTION ADMINISTERED INTO PERITONEAL CAVITY AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN THE BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
15	50 cc. of 1 per cent solution	0.14	0.14	0.16		0.16	30	31	32		30
16	100 cc. of 0.5 per cent solution	0.13	0.15	0.17		0.17	35	32	36		37
35	100 cc. of 0.4 per cent solution	0.15	0.15	0.16	0.17	0.19	16	16	16	17	16
36	50 cc. of 0.4 per cent solution	0.14	0.17	0.17	0.17	0.16	19	23	22	22	21
38	50 cc. of 0.4 per cent solution	0.14	0.14	0.16	0.18	0.20	21	23	23	21	22

The effect of HCl *solution by mouth on the blood diastase.* Acid introduction or acid production in the body exerts a distinct influence on carbohydrate metabolism.

Elias (25) states that introduction of acids into dogs and rabbits leads to hyperglycemia and glycosuria. In this investigation we only followed the changes in the blood for four hours after the ingestion of acid, and this may not be long enough to show the influence of this drug. In some cases a rise in the blood sugar was noted one hour after

TABLE 6

The sugar and the diastatic activity of the blood before and after administration of HCl solution by mouth

RABBIT NO.	HCl SOLUTION BY MOUTH AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
42	100 cc. of 0.5 per cent solution	0.14	0.16	0.14	0.16	0.17	23	22	23	26	23
43	100 cc. of 0.8 per cent solution	0.16	0.21	0.18	0.18	0.17	22	25	24	26	27
48	100 cc. of 1 per cent solution	0.16	0.21	0.17	0.17	0.16	28	28	25	27	28
49	100 cc. of 1 per cent solution	0.14	0.20	0.18	0.19	0.19	23	24	24	25	25
44	150 cc. of 1.36 per cent solution	0.16	0.17	0.16	0.17	0.18	26	26	25	25	26

ingestion of HCl, but the results were not uniform. We do not consider this rise as being due to the influence of the acid. The HCl has no noteworthy effect on the diastase content of the blood, with the exception of rabbit 43 which shows a slight rise in diastase.

The effect of the injection of epinephrin upon blood diastase. Blum (26) in 1901 discovered that adrenal extract injected subcutaneously gives rise to glycosuria. Early in 1902 the discovery was made by Herter and Richards (27) that the injection of a solution of adrenalin chloride into the peritoneal cavity of dogs was followed by an intense though transient experimental glycosuria. Herter and Wakeman (28) also reported experimental glycosuria by epinephrin injection. Metzger (29) proved definitely that the glycosuria resulted from hyperglycemia.

TABLE 7

The sugar and the diastatic activity of the blood before and after injection of adrenalin

RABBIT NO.	ADRENALIN SUBCUTANEOUS INJECTION AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
20	0.5 cc. per kilo	0.16	0.55	0.71		0.56	37	41	35		37
50	0.5 cc. per kilo	0.15	0.43	0.52	0.47	0.38	27	31	30	29	30
21	0.5 cc. per kilo	0.17	0.51	0.54	0.58	0.62	29	31	32	28	28
34	0.4 cc. to 1350 grams rabbit	0.17	0.24	0.22	0.20	0.18	20	21	18	20	20

Vosburgh and Richards (30) have made a somewhat detailed study of the sugar in the blood after the intravenous injection of adrenalin as well as after application of that substance to the pancreas. They noted that the glycosuria provoked by epinephrin was due to an increase of sugar in the blood. Later many investigators proved that the increased sugar came from the liver glycogen.

Starkenstein (31) found no appreciable difference in the diastase content after using adrenalin piqûre and various other procedures. Allen (32) stated that adrenalin, phlorizin, phloretin and asphyxia have no effect on blood diastase.

In our investigation marked hyperglycemia and glycosuria appeared, but the diastase content of the blood was not affected.

Besides the above experiments, two rabbits were injected repeatedly with 0.5 cc. to 1.0 cc. epinephrin during a period of from ten to eighteen hours. There was no effect on the diastase content but there appeared

a decrease in blood sugar after successive injections. The diastase content and blood sugar increased markedly before death in a rabbit injected with 1 cc. epinephrin.

The effect of ether anesthesia on the concentration of blood diastase. Hawk (33) and Seeling (34) noted that glycosuria appeared after ether anesthesia; other anesthetics also possess the power to induce hyperglycemia, and at the same time glycosuria. Carlson and Luckhardt (8) found a decreased diastatic activity during ether anesthesia. Blood was drawn during anesthesia which varied from twenty minutes to two hours. Bloods taken before and after anesthesia were compared and the serum drawn during anesthesia had a lesser diastatic activity than the normal serum, but this variation is not much greater than is exhibited in normal animals. They attribute this phenomenon to

TABLE 8

The sugar and the diastatic activity of the blood before and after ether anesthesia

RABBIT NO.	PERCENTAGE OF BLOOD SUGAR			DIASTATIC ACTIVITY IN BLOOD		
	8.55 a.m.	10 a.m.	11 a.m.	8.55 a.m.	10 a.m.	11 a.m.
30	0.14	0.22	0.18	29	32	28
31	0.16	0.25	0.20	25	24	22
32	0.14	0.34	0.18	26	29	25
33	0.16	0.26	0.16	28	32	28
Average	0.15	0.27	0.18	27	29	26

the inhibitory action of the anesthetic on the enzyme since there is an increased diastase production during anesthesia. In similar experiments Ross and McGuigan (35) obtained negative results. Carlson and Ryan (36) reported that during anesthesia cat's saliva contains more diastase than usual, this being due to increased concentration of the blood diastase. Carlson and Luckhardt determined the amount of diastase (1) by the rate of clearing, and (2) by the rate of the complete disappearance of erythrodextrin in starch solution.

Four rabbits were anesthetized for exactly one hour in our experiments, and the activity of the blood diastase was compared just before and after and also one hour following the anesthesia.

All rabbits showed hyperglycemia just after anesthesia. The diastase was increased slightly but not markedly on the average, and this increase cannot be considered due to the influence of ether. The blood drawn one hour after anesthesia showed a slight decrease in diastase content.

The effect of the administration of pituitrin on blood diastase. Pituitrin has no important influence on carbohydrate metabolism. In this experiment tablets were administrated by mouth in two cases and pituitrin (Parke-Davis) was injected subcutaneously in two other cases. There was no change in the blood diastase, but the blood sugar increased, especially in experiment 24. This phenomenon may be explained by

TABLE 9

The sugar and the diastatic activity of the blood before and after administration of pituitrin

RABBIT NO.	PITUITRIN ADMINISTRATIONAT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
22	Gr. III tablet by mouth.....	0.17	0.22	0.19	0.18	0.19	33	31	32	32	31
23	Gr. III tablet by mouth.....	0.14	0.14	0.14	0.16	0.16	35	33	35	36	37
24	0.25 cc. injection into subcutan...	0.15	0.22	0.18	0.24	0.25	23	23	24	22	22
25	0.4. cc. injection into subcutan...	0.17	0.21	0.17	0.18	0.18	26	26	26	25	24

TABLE 10

The sugar and the diastatic activity of the blood before and after feeding thyroid tablet

RABBIT NO.	THYROID TABLET EACH TABLET = GR. I OF EXTRACT OR = THYROIDS GR. V FRESH SUBSTANCE AT 9 A.M.	PERCENTAGE OF BLOOD SUGAR					DIASTATIC ACTIVITY IN BLOOD				
		8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.	8.55 a.m.	10 a.m.	11 a.m.	12 m.	1 p.m.
26	One tablet	0.16	0.16	0.17	0.18	0.18	26	26	26	27	26
27	One tablet	0.13	0.14	0.13	0.13	0.14	30	28	26	26	26
28	One tablet	0.17	0.17	0.16	0.20	0.19	29	31	28	27	29
29	One tablet	0.13	0.14	0.15	0.15	0.15	26	24	25	29	27
Average.....	One tablet	0.15	0.15	0.15	0.17	0.17	28	27	26	27	27

the fact that there is a concentration of the blood, the rabbits excreting large volumes of urine after the administration of pituitrin.

The effect of ingestion of thyroid tablets upon the blood diastase. Flesch (37) found that thyroid feeding and transplantation of the thyroid gland caused alimentary hyperglycemia. Cramer and Krause (38) reported that the glycogen content of the liver was decreased to a minimum after thyroid feeding in rabbits and rats. In dogs the toler-

ance for glucose was distinctly lowered, and they concluded that thyroid feeding inhibited the formation and storage of glycogen in the liver. Carmer and McCall (39) noted that the thyroid hormone stimulated the oxidation of glycogen and this explains the increased oxidation of carbohydrates. Recently, Kuriyama (40) stated that experimental hyperthyroidism induced by thyroid feeding produced neither a change in the blood sugar nor spontaneous glycosuria in rabbits or rats.

In this investigation tablets were given by mouth and results were followed during the first four hours after administration. This length of times does not seem sufficient to affect the body metabolism. However, if there were any changes in the blood diastase, it would at least occur in the last specimens drawn, but no changes appeared in blood sugar or diastase.

SUMMARY AND CONCLUSIONS

1. The intravenous injection of human saliva in rabbits causes an increase in the amylolytic activity of the blood.

2. The parenteral administration of soluble starch increases slightly the diastase of the blood and causes an elimination of amylase in urine a few days after the injection. Injection of large doses does not produce a sudden change in the blood but causes a successive increase over a period of several days.

3. The intravenous injection of $NaHCO_3$ (5 or 10 grams) shows a slight increase in blood diastase and sugar. When the above dose was injected intraperitoneally, a marked hyperglycemia and an increase of diastase occurred. The injection of the above dose into the peritoneal cavity caused the death of the rabbits.

4. The injection of smaller doses of Na_2CO_3 intraperitoneally had no effect on the blood diastase or sugar.

5. No marked changes in the blood diastase and sugar appeared within four hours after the ingestion of HCl.

6. The subcutaneous injection of epinephrin causes hyperglycemia and glycosuria but produces no appreciable change in the blood diastase. Intravenous injection causes hyperglycemia and at the same time an increased amylase in a fatal case.

7. Ether anesthesia is followed by hyperglycemia but the diastase remains practically constant except for a slight tendency to increase immediately after the anesthesia.

8. Pituitrin injection or ingestion has no effect on the blood diastase or sugar. The increased sugar content observed was probably due to a

concentration of blood, caused by an excessive excretion of urine after the administration of the pituitrin.

9. Thyroid feeding has no appreciable effect on the sugar and diastase content of the blood within four hours after feeding.

The author acknowledges his indebtedness to Prof. V. C. Myers for his suggestions concerning this work.

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THE DIFFERENTIATION OF THE MINIMAL CONTRACTION IN SKELETAL MUSCLE

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With all the work published on the activity of skeletal muscle and the very frequent use of the terms *threshold* and *minimal*, there has been little if any investigation to determine directly the character of the true minimal contraction. This is doubtless owing to several reasons. In the first place, muscle has been worked upon as a continuous system, responsive in imperceptible degrees of difference to continuously varying strength of stimulus. The absolute minimal has been an ideal, infinitesimal in value and hence indeterminable by observation. In the second place, an arbitrary standard—the least perceptible contraction—has been set up, and found adequate in practice for determinations of threshold. In the light of the all-or-none principle, however, the way is open for more definite analysis which, in its turn, will further test the validity of that principle. Moreover, since the motor nerve fiber is known to actuate a multiple contractile system, the true minimal is to be sought not by indirect but by direct excitation of the unit components of the system. In order, therefore, to generalize concerning fundamental muscle activity it is not sufficient to accept conclusions drawn from the behavior of the muscle as a whole. A clear dynamic conception must be brought one step nearer by the study of the single element. Such a study has been made possible by the development in this laboratory of the capillary pore electrode, through the use of which the results here presented have been secured.

It seems probable that the smallest muscular unit capable of individual activity is the fiber. That this activity is, under varying strength of stimulus, fixed, is further to be regarded as probable (1), (2). In the directly excited muscle, therefore, it should be possible by sufficiently local stimulation to reveal a response of definite, irreducible value. Such a response should prove to be that of a single fiber.

In the course of many experiments in this laboratory, utilizing the above-mentioned apparatus, a small group of muscle fibers (often apparently a single fiber) was observed to glide independently among its neighbors, drawing upon the latter as passive structures. Again, quite frequently, a straight capillary running across the fibers would be drawn to a marked angle by the action of the fiber or fibers beneath. Such observations have led to the definite attempt, reported in this paper, to identify the minimal contraction with the functionally isolated fiber. It has recently been shown by Pratt (2) that the gradients of varying activity characteristic of skeletal muscle, including those of fatigue and staircase, may exhibit in common an all-or-none or "quantal" constitution. The work here set forth is designed to bring further light to bear upon the basic unit concerned in such effects, revealing it to direct observation.

METHODS

The apparatus for the direct stimulation of the muscle fiber is, with slight change in form but not in principle, that described by Pratt (3). The modification has been essentially for the purpose of permitting observation of the contracting element up to within two or three millimeters of the point of stimulation, and is shown in figure 1, with the omission of details of support, etc. It will be noted that the physiological solution covering the preparation is made to serve as the indifferent pole. The muscle used is the sartorius of a surviving preparation —a pithed leopard frog (R. pipiens) of medium size, uncurarized, with circulation intact. The muscle is exposed by cutting and laying back the skin on the ventral surface of the thigh. Both sides are prepared at the same time for convenience in exposing the entire length of both sartorius muscles. With the frog placed on its back and the solution just covering the bared tissues, a low-power objective can easily be brought to focus upon any portion of the muscle. In such a preparation the capillary circulation is readily observed throughout an experiment, frequently as long as six to eight hours. It is often at first very rapid, while later it may diminish considerably; the individual corpuscles may then be easily distinguished even with the direct illumination necessarily used. The illuminating system consists of two parts; one of moderate intensity for direct visual observation, and the other a very intense white spot-light produced by focussing the rays from a twelve candle-power tungsten bulb through a two-thirds microscopic objective—a method similar to that devised by Patten (4) for localized

stimulation by light. This, or a similar white light directly applied, has been used for most of the photographic records. These are secured by drawing a photographic plate over a suitable shutter arrangement placed over the ocular of the microscope, which retains its usual system of lenses. The moving mechanism consists of a plate-holder gliding very smoothly over glass slides and drawn by the spindle of a kymograph. This arrangement permits of any rate of movement or frequency of exposure needed. Records are traced, as in figure 3, by the light reflected from minute globules of mercury sprayed under high pressure from a fine orifice at the end of a glass tube. A globule,

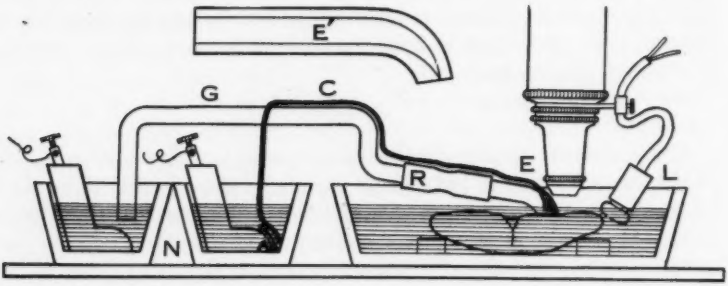

Fig. 1. Modification of the capillary pore electrode and accessory parts; preparation immersed in a physiological solution. *N*, baths of same solution, with non-polarizable boot-electrodes (Porter); *G*, glass tube supporting pore-bearing tube, *E*, and filled with the solution; *R*, rubber connection, permitting delicate and yielding pressure of electrode upon tissue; *C*, narrow strip of chamois, saturated with the solution and forming, with the liquid in contact with the preparation, the indifferent terminal; *E'*, section (actual size) of the pore-bearing tube—the active terminal.

resting immediately over the active region of the muscle and separated from it only by a very delicate fascia, moves in a majority of cases exactly with the contracting fiber or fibers. In a few instances where the globule movement is not so great as that of the fiber, owing probably to heavier fascia, the movement is strictly relative as observed with the ocular micrometer.

Tetanizing stimuli are produced by the magneto-inductor previously used in this laboratory (2) giving 50 to 60 alternations of current per second, with the intensity under very delicate control by the operator at any instant. Twitches of muscle fibers are made with induced break shocks only. The primary circuit of the inductorium, with a

single Daniell cell, includes a large straight rheocord serving two purposes: first, to reduce greatly the sparking at the platinum-mercury key; and second, to control delicately the intensity of the stimuli.

The procedure in most experiments is to apply the electrode to the surface of the sartorius at about the junction of the middle and distal thirds, shifting the electrode until a suitable contracting element can be observed. It is not difficult to select a fiber out of a very small group in any location by simply sliding the electrode carefully across the fibers by means of the horizontal adjustment. In the sartorius, however, which is generally supposed to contain a majority of long, straight fibers, it is found that many fibers which lie superficially at the point of stimulation are very shortly buried beneath the surface, many times not to reappear. The region of stimulation, distal, was first selected because of its presumable freedom from nerve filaments. However, there has never been any difficulty in avoiding indirect stimulation, even in other locations. A number of experiments have been made with the electrode applied to the pelvic end of the muscle. This is a more difficult procedure, owing to the tilt of the surface in this location, but the results have been equally good in every respect. In some experiments, where the extent of contraction has been rather limited, a greater movement has been obtained by cutting the surface fibers at one or the other end of the muscle, together with the overlying fascia. The resulting difference is that excursion is increased, with active and idle fibers more readily distinguished. The muscle may be further relaxed by flexing the leg on the thigh.

Observations are made by watching with the micrometer ocular the movement of the mercury globule, or that of a minute speck of carbon blown on the surface of the preparation; or, finally, by observing directly the movement of small capillaries that chance to cross the particular element under observation, and themselves frequently serve as clear indices for scale reading. For the purpose of this paper the muscle fibers themselves have to a large extent been observed directly with the microscope. The average width of a fiber as exposed on the surface is about 40 μ in the medium sized specimens used.

The stimulating current is always of very low intensity. The full capacity of the several generating apparatus, just perceptible to the tip of the tongue, is never used; and even further decrement must result from the high resistance at the electrode pore. An attempt has been made several times to use a pore of 3 μ diameter without success; apparently the full current was insufficient to pass so fine an orifice

and still have sufficient intensity to stimulate a fiber. The electrode now in use has a very clean-cut circular pore of 7 μ diameter; it seldom becomes plugged in use, and is of the form shown in figure 1, E and E'.

To enable localized activity to reveal itself the more distinctly by photographic recording, the following method is used. The frog is prepared as usual and the sartorius tibial tendon carefully cut without other injury to the muscle. This leaves the circulation intact and permits the surface fibers to relax. The source of illumination is placed on a level with the fibers to be observed, thus showing them in relief. The stimulating electrode is placed at about the middle of the sartorius, while the record is taken near the tibial end of the muscle where the relaxation of the fibers is greatest.

For the recognition of a minimal effect, the appearance of the activity of a single fiber is not alone sufficient; further tests must be applied. The apparent fiber must not be capable of variable response or give any evidence of a possible smaller contraction. This is determined in two ways. First, the strength of the stimulus is repeatedly increased and decreased very gradually above and below the apparent threshold. Often a very marked alteration of coil position may be made without changing the response from its uniform character. If no smaller step is in this way differentiated, a second test is used if any doubt exists. A uniform stimulation series for the apparent minimal response is maintained until the rising threshold in fatigue induces relaxation. If this occur in a single step, the test is considered satisfactory. If it take place in two or more steps, it shows the probability of being caused by the elimination of more than one fiber (2). The last step is here found by test to be all-or-none, and the single active fiber can usually be satisfactorily observed unless it has ceased to be superficial at the point of observation. Under such conditions the activity, even though it be of a single fiber, will impart a general passive movement to the fibers in that location. This movement will also be found to be all-or-none in character.

OBSERVATIONS AND EXPERIMENTAL RESULTS

The four drawings in figure 2 illustrate the test above mentioned, and are made from sketches secured during observations in an early experiment. In this experiment the pore of the electrode used was about 35 μ in diameter, much larger than that subsequently employed. Views A and B are of the same portion of a field at rest and active.

The illumination used was medium in intensity and produced, at the depression of sartorius surface along the edge of the rectus abdominis, a linear reflection of light expressed in the drawings as a broad line. The distortion of this line, as shown in drawing *B*, from tension directed nearly at a right angle, would suggest a very limited activity, if not that of one fiber. On further test, however, as shown in views *C* and *D*, more than one unit could be differentiated. In these two latter views (the same field as of the former) the illumination was greatly increased and was directed from a different point, giving the multiple reflections as shown. The same current strength was applied for both *B* and *D*;

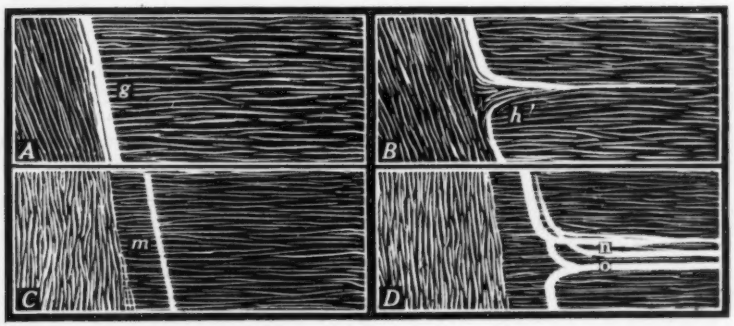

Fig. 2. Experiment of September 6, 1916. *A*, the preparation at rest; *g*, linear reflection at margin of rectus abdominis. *B*, the preparation stimulated; *h*, distorted reflection from local tension in sartorius. *C*, at rest under altered illumination; *m*, marginal reflection. *D*, again stimulated; *n*, tension lines eliminated in fatigue; *o*, residual line of minimal tension. The letters, *A*, *B*, *C*, *D*, rest upon the rectus abdominis. Further description in the text.

the difference appeared only on change of illumination. Next, the stimulus was applied continuously until the contracting field relaxed. The activity did not, however, end in one abrupt relaxation, but first *n* suddenly yielded, followed several seconds later by *o*, showing definitely that what had at first appeared as the possible activity of a single fiber was, in reality, probably that of two.

Figure 3 shows photographically the record of reflection from two globules of mercury on the surface of the sartorius. Five muscle fibers intervened between them. This interval was approximately 200 μ, or $\frac{1}{5}$ mm. The electrode stimulated the fiber beneath the upper globule, which traced twitches of two heights or steps; the higher contraction

approximately $\frac{1}{50}$ mm; and the lower, $\frac{1}{150}$ mm. The muscle fibers crossed at right angles to the abscissa. It will be noted that the lower tracing shows no interruption corresponding to the contractions, thereby indicating the very limited area of activity with stimulation by this method. By direct observation with the high-power ocular the same result was apparent; the second globule did not move even with the higher response produced by more than one fiber. This

Fig. 3. Experiment A, August 15, 1917. Photomicrographic tracing (negative) from two mercury globules on the surface of the sartorius, reading from left to right. The record was made on a uniformly moving plate. Description in the text.

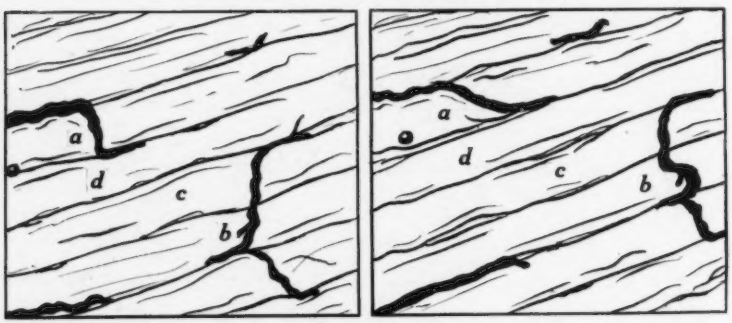

Fig. 4. Experiment A, August 2, 1917. Described in the text.

figure also illustrates the method of test for determining the minimal step by the fatigue process. The twitches were produced by the apparent minimal stimulus, using a Daniell cell with rheocord resistance. The strength of the stimulus remained constant, and was applied every two seconds. The first step, apparently minimal when the experiment began, had soon differentiated from it a lower step which on further observation proved to be all-or-none—the true minimal.

The drawings in figure 4 show the possible activity of two adjoining

fibers, both stimulated by the electrode at the point of application but separated by an intervening fiber in the field of observation. At the right, capillary *a* is pulled to a sharper angle by the contraction of fiber *d*. Capillary *b* shows a distortion to the right of the letter, from the pull of the fiber beneath. A passive fiber, *c*, lies between. At the left the muscle is at rest. An instance such as this is often encountered

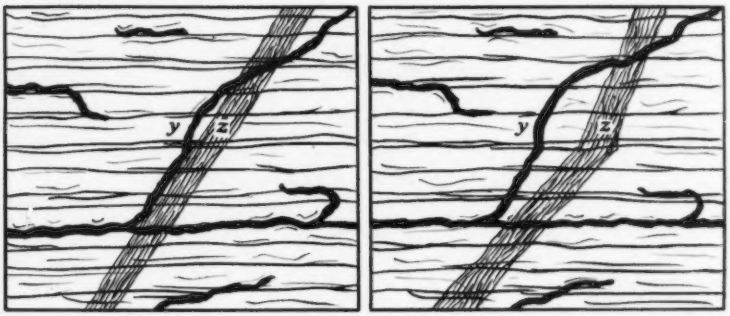

Fig. 5. Experiment of June 14, 1917. Described in the text.

Fig. 6. Experiment of August 28, 1916. Described in the text.

when applying the electrode near the tibial end of the muscle where the fibers rapidly converge to blend with the tendon. Some of the fibers disappear beneath their neighbors to attach to the tendon deeply. Thus, a fiber appearing on the surface at the central end may not be in the field of stimulation at the tendon end. The reverse of this may also be found.

In figure 5 is seen the effect of stimulating a fiber which dips below the surface; z being a deep vessel, y a surface capillary which remains stationary. The vessel, z, is drawn to a marked angle by the hidden contracting element.

Figure 6 presents the reverse phenomenon. A surface fiber is stimulated; the deep vessel remains fixed. It is evident that the sharp angle to which the originally vertical capillary is drawn, the expansion of the capillary at e from stasis, and also the changed direction of the blood-stream as indicated by the arrows, are the result of sharply localized fiber activity.

In figure 7, at the left, is shown the muscle surface at rest with all the fibers relaxed. A deep vessel lies at v. On the right is the same

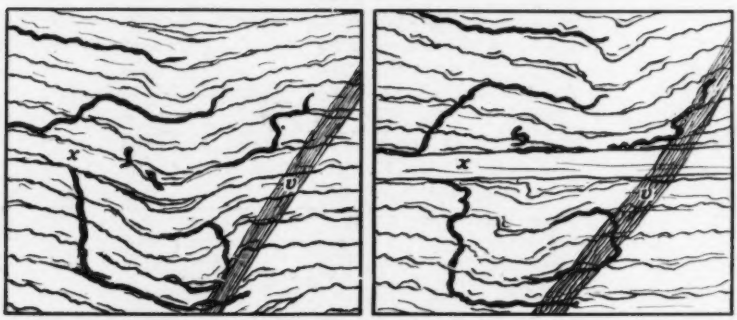

Fig. 7. Experiment of September 17, 1917. Described in the text.

field with the fiber, x, active. Note the tense appearance of x; the slight pull on the deep vessel, v; the changed relative positions of the surface capillaries. This is from a careful sketch from actual observation at the microscope. The threshold in this preparation altered rapidly and it was impossible to secure a good photographic record. The strength of stimulus was changed several times during the sketching to maintain the minimal response.

Figure 8, a photograph, shows at the left the muscle surface at rest. The fiber to contract is marginally indicated as x. The position and angle of the two capillaries crossing this fiber should be noted, as well as the heavy capillary loop, a. The latter remains stationary. At the right is the same field with the fiber, x, in a state of contraction. The abscissa is preserved in a bright spot beneath each globule; the

excursion of the lower globule is, in fact, delicately traced by this point of highest light-intensity. Note the distinctly altered angles of the capillaries crossing *x*, with the passive change as they cross the fibers to the left. The capillary, *a*, remains stationary, although the movement of the mercury globule shows that the fascia upon which it rests is decidedly deformed by the contraction of *x*. By direct observation the fiber, *x*, could be distinctly seen to slide past all its neighbors with a greater freedom of movement.

Figure 9 shows, at the left, the muscle surface at rest. The fibers are nearly straight and not particularly distinct. Capillary *a* is fairly straight. The arch of capillary *c* is quite distinct, crossing two fibers.

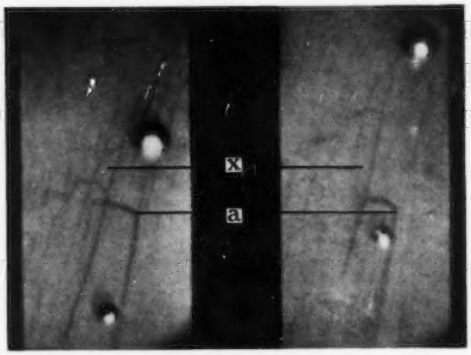

Fig. 8. Experiment B, August 2, 1917. Described in the text.

At the right is the same field showing the activity of fiber *x*, which now appears more tense and even straighter than at first, with a slight general movement of the field downward. The fibers on either side of the tense, active fiber have a crumpled appearance very characteristic of idling elements in a relaxed muscle. Capillary *a* is now wavy in form. Capillary *c*, drawn slightly out of focus, still retains practically the same arched contour, showing that the two fibers which it crosses, so decidedly crumpled, still retain their relative position to each other and are not themselves active.

Figure 10 shows at the left the muscle surface at rest, with all the fibers in a relaxed, crumpled position. At the right the fiber, *x*, is in a state of contraction; it appears quite tense and straight. The other fibers are still relaxed. This observation is near the cut tendon, and,

as a result of the contraction, most of the field is drawn more or less out of focus.

Figures 11 and 12 both show the same characteristics: the crumpled appearance of the fibers at rest; in contrast the tense appearance of the contracting fiber x; and the changed relative positions of prominent capillaries.

In all of these photographic records the tests mentioned earlier in this paper were used to differentiate the minimal contraction. However clear these minimal effects may appear as figured, it is entirely impossible for them to impart the conviction, afforded by even one direct visual observation, that the observer is witnessing the absolute minimal

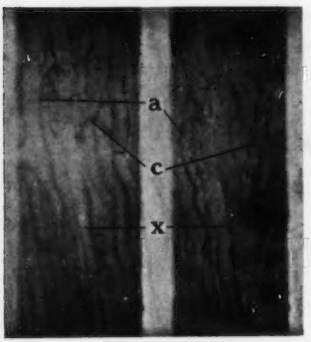

Fig. 9. Experiment C, August 16, 1917. Described in the text.

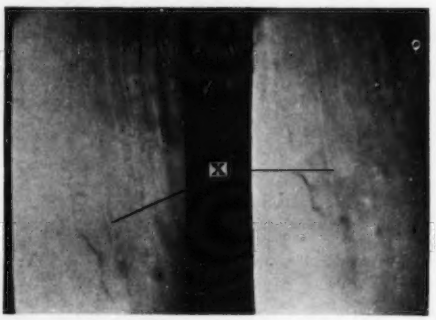

Fig. 10. Experiment C, September 17, 1917. Described in the text.

response of a muscle—a single fiber, at the will of the operator, gliding smoothly among its neighbors in a living preparation. The use of the capillaries with their changed relative positions is only a convenient aid to show the definitely limited character of the response to stimulation; filled with the circulating blood, they produce sufficient color-contrast with the fibers to record readily on the photographic plate. The capillaries, indeed, often outline the individual fibers. But by direct observation the fiber substance itself may be distinctly seen to move with a swift caterpillar-like effect of shortening, while adjoining fibers lie passive.

On consideration of the details of the method—in particular, the circulation-preparation, the width of the exposed muscle fiber as against the small diameter of the stimulating pore, and the very minute strength

of the exciting current with its probable extremely local effect (3)—it would seem that conditions have been realized highly favorable to the isolated response of a single fiber. It has been shown that in many cases the response, under direct observation, is plainly that of a single

Fig. 11. Experiment of September 18, 1917. Described in the text.

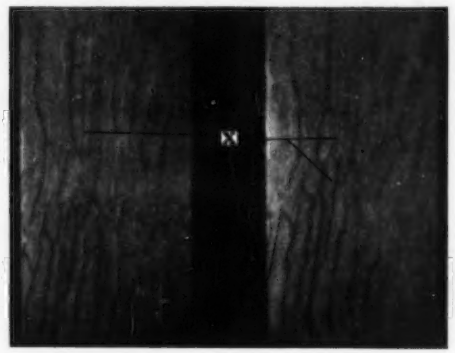

Fig. 12. Experiment of September 20, 1917. Described in the text.

fiber. In cases of doubt, even where change of stimulation strength appears to leave the extent of contraction unaffected, the minimal effect may be differentiated by elimination through the fatigue process. Thus it comes about that even when stimulation, through spread of current, is relatively diffuse, the falling out of successive contractile

units as fatigue manifests itself will tend finally to leave one element "standing." Such a remainder, as I have endeavored to show, is the fiber. Its contraction is of unit value, holding its own through rising threshold or falling stimulus until it, likewise, is abruptly degraded to zero. The minimal contraction is not an arbitrary term; nor does it reflect the abstract ideal attaching to a continuous system. It is a concrete event, readily elicitable by appropriate excitation—visible and capable of measurement.

SUMMARY

1. This paper reports a method for revealing a true minimal entity in the contraction of directly stimulated skeletal muscle. The existence of such an entity is implied in the all-or-none principle of Keith Lucas. Its determination as an observed phenomenon must serve as a further support of that principle and render possible various observations foreign to the study of the muscle as a whole.

2. The sartorius muscle *in situ* of the pithed frog, uncurarized, with intact circulation is used in all experiments. The stimulating apparatus consists in the capillary pore electrode and its accessory devices, with appropriate modifications. Microscopic and photographic apparatus for the purpose of observing and in various ways recording localized fiber activity are described.

3. The procedures involve: observation of local contraction; application of tests for minimal effect; verification, by observation and photographic record, of the uni-fibral origin of an irreducible contraction.

4. A number of drawings and photomicrographs are presented, showing the activity of single fibers, and the minimal function of these is supported by experimental evidence.

My thanks are due to Dr. Frederick H. Pratt for kindly direction during the course of this investigation.

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THE EFFECT OF ALCOHOLIC INTOXICATION ON CATALASE

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The literature on the subject of alcohol is so large and in many respects so conflicting that no attempt will be made to review it.

We have shown that the catalase of the blood is increased during the stimulating stage of narcosis and decreased during deep narcosis. It was also shown that the increase in catalase was due to an increased output from the liver and that the decrease during deep narcosis was due to a destruction of the catalase by the narcotic. If it is true that catalase is the enzyme in the animal organism principally responsible for oxidation, possibly in the manner suggested by Bach and Chodat, then it may be that the decrease in catalase with resulting decrease in oxidation is the cause of deep narcosis, while the increase in catalase with the resulting increase in oxidation is the cause of the excitement stage. It seems to be a disputed question as to whether alcohol produces a stimulating effect or a depressing effect. If it can be shown that the initial effect of alcohol is to increase the catalase of the blood and hence of the tissues and that during alcoholic coma catalase is decreased, this would seem to argue that the initial effect of alcohol is to stimulate while the remaining effect is to depress. The object of this investigation was to determine the effect of alcoholic intoxication on the catalase content of the blood. Dogs were used in the experiments. Previous to the introduction of alcohol the animals were slightly etherized and during this period of slight anesthesia at least two determinations were made to see that the catalase of the blood had become constant. When this was found to be the case these determinations were taken for comparison as the normal catalase of the blood. Alcohol was then introduced and after this had taken effect, which required as a rule fifteen or twenty minutes, the administration of ether was discontinued. At intervals of fifteen minutes samples of blood were taken and the catalase content determined. The determinations were made by adding 0.5 cc. of blood to 50 cc. of hydrogen

peroxide in a bottle at 22°C. and as the oxygen gas was liberated, it was conducted through a rubber tube to an inverted burette previously filled with water. After the volume of gas thus collected had been

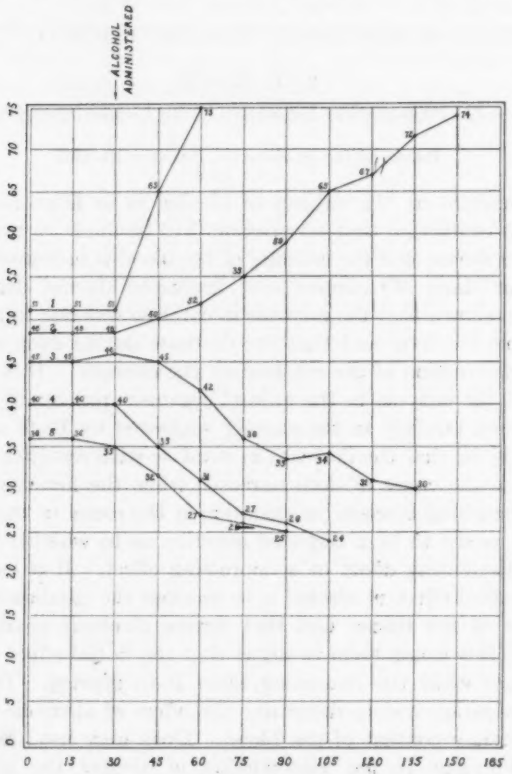

Fig. 1. Curves showing effect of alcohol on the catalase content of the blood. The figures along the abscissa (0 to 165) indicate time in minutes, while the figures along the ordinate (0 to 75) represent amounts of catalase indicated in cubic centimeters of oxygen liberated from hydrogen peroxide in ten minutes by 0.5 cc. of blood.

reduced to standard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 0.5 cc. of blood.

Curve 1 was constructed from data obtained from a dog after the introduction of 150 cc. of 60 per cent ethyl alcohol into the stomach of

the animal. It will be seen that 0.5 cc. of the samples of blood taken during the thirty-minute interval at the beginning of the experiment and before any alcohol had been introduced into the stomach of the animal, liberated 51 cc. and 51 cc. of oxygen respectively from hydrogen peroxide in ten minutes; that fifteen minutes after the alcohol had been introduced or at the end of the forty-five minute period, 0.5 cc. of the blood liberated 65 cc. of oxygen, while thirty minutes after the introduction of the alcohol, it liberated 75 cc. of oxygen. By comparing the amount of oxygen liberated by the blood previous to the introduction of alcohol with that after the introduction of alcohol, it will be seen that the amount of oxygen had increased from 51 to 75 cc. Curve 2 was constructed from data obtained from a dog after the introduction of 20 cc. of 30 per cent ethyl alcohol at intervals of thirty minutes. It will be seen that 0.5 cc. of blood of the two samples taken previous to the introduction of alcohol liberated 48 and 48 cc. of oxygen respectively from hydrogen peroxide; that after the introduction of alcohol the amount of oxygen liberated increased from 48 cc., the normal, to 74 cc., the amount liberated by the sample of blood after the dog had been under the influence of alcohol for one hundred and thirty-five minutes.

The question that naturally arises in this connection is, how does the introduction of alcohol into the stomach bring about an increase in the catalase of the blood? Since the alcohol is absorbed directly from the alimentary tract into the blood, the answer that suggests itself is that the increased catalase may be due to the direct action of the alcohol on the blood, or to the stimulating effect of the alcohol on the liver thus increasing the output of catalase from this organ.

The following experiment was carried out to determine if the increase in catalase was due to the direct action of the alcohol on the blood. To 18 cc. of defibrinated dog's blood 2 cc. of 95 per cent ethyl alcohol were added. The data from which curve 4 was constructed were obtained from determinations of the catalase of this blood. Two determinations of the catalase of 0.5 cc. of the blood were made previous to the addition of the alcohol, while the remaining determinations were made at intervals of fifteen minutes after the addition of the alcohol. It will be seen that 0.5 cc. of the blood before the addition of the alcohol, liberated 40 cc. and 40 cc. of oxygen respectively; that after the addition of alcohol, the catalase of the blood decreased as is indicated by a decrease from 40 cc. of oxygen, the amount liberated previous to the addition of alcohol, to 24 cc. of oxygen, the amount liberated by 0.5

cc. of the blood ninety minutes after the addition of the alcohol. This observation is interpreted to mean that alcohol of this concentration destroys the catalase of the blood. It might be said in this connection that several other experiments were carried out using much weaker as well as much greater concentrations of alcohol, and it was found that the effect of alcohol was to destroy the catalase, the rate of destruction being faster the greater the concentration.

The data for curve 3 were obtained from a dog into whose jugular vein 40 per cent ethyl alcohol was introduced at the rate of approximately 1 cc. per minute. Previous to the introduction of the alcohol, the catalase content of two samples of the blood was determined. It will be seen that 0.5 cc. of these samples liberated 45 cc. and 46 cc. oxygen respectively from hydrogen peroxide in ten minutes. Fifteen minutes after the introduction of alcohol into the blood, a determination of the catalase content of 0.5 cc. was made. Similarly determinations were made after thirty, forty-five, sixty, seventy-five, ninety, one hundred and five, and one hundred and twenty minutes respectively. It will be seen that the introduction of alcohol into the blood through the jugular vein decreased the catalase of the blood after two hours by 33 per cent as is indicated by a decrease in the amount of oxygen liberated from 45 to 30 cc. This observation is interpreted to mean that the introduction of alcohol into the blood vessels of the living animal destroys the catalase of the blood.

The question why the introduction of alcohol into the blood from the alimentary tract should increase the catalase, while the introduction of alcohol directly into the blood vessels should decrease the catalase, naturally arises. One answer that suggests itself is that when the alcohol is absorbed from the alimentary tract and is carried directly to the liver, it may stimulate this organ to an increased output of catalase. If this explanation is the correct one, then the introduction of alcohol into the portal blood should increase the output of catalase from the liver. Accordingly, experiments were carried out to see if this were true. By means of a hypodermic needle attached to a burette by a piece of rubber tube, 40 per cent ethyl alcohol was introduced into the portal blood at the rate of approximately 1 cc. per minute while the catalase of the blood taken from the external jugular was determined at intervals of fifteen minutes. For comparison the catalase of the blood was determined after fifteen and thirty minute intervals at the beginning of the experiment before the introduction of the alcohol. It will be seen in curve 5 that 0.5 cc. of these samples of blood liberated

36 and 35 cc. of oxygen in ten minutes. After the thirty-minute interval, the alcohol was injected at the rate stated. It will be seen that the effect of the injection into the portal vein was not to increase the catalase of the blood as was expected, but to decrease it as is indicated by the decrease in the amount of oxygen liberated by 0.5 cc. of the different samples of blood. Why the absorption of alcohol from the alimentary tract should produce an increase in the catalase of the blood, while the introduction of alcohol into the portal vein produced a decrease, I am not as yet prepared to state.

As a result of the experiments reported in this paper, it is assumed that in so far as the absorption of alcohol from the alimentary tract produces an increase in the catalase of the blood resulting presumably in an increase in oxidation, just so far alcohol exerts a stimulating effect, while in so far as the accumulation of alcohol in the blood in prolonged intoxication or the introduction of alcohol directly into the blood destroys catalase, resulting presumably in a decrease in oxidation, just so far alcohol exerts a depressing effect.

SUMMARY

The introduction of alcohol into the stomach greatly increases the catalase of the blood, while the introduction of alcohol directly into the vascular system decreases the catalase of the blood. The decrease in catalase produced by the introduction of alcohol directly into the blood is due to the destruction of the catalase by the alcohol. Further work is necessary before an explanation can be given for the increase in the catalase of the blood when alcohol is absorbed from the alimentary tract into the blood.

HAEMODYNAMICAL STUDIES

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I. THE BLOODFLOW DURING IMMERSION IN COLD WATER

These experiments purposing to show the influence of immersion in cold water upon the flow of the blood, were performed upon small dogs during light ether narcosis. In each case the animal was placed in a small bath tub with the shoulders and head elevated in such a way that the body could be covered later on with water without interfering with respiration. A stromuhr[1] was then inserted in the left common carotid artery. The central cannula of this instrument was connected with a mercury manometer for the registration of the general arterial pressure. The respiratory movements were recorded by means of a simple stethographic arrangement.

The procedure followed in these experiments consisted in the determination of the bloodflow under normal conditions and during the immersion of the animal in water of 25 to 32°C. Lower temperatures than these were not employed, because the muscular reactions then usually resulting, tended to increase the general blood pressure very abruptly, and also interfered with the registration of the bloodflow in a mechanical way. I have sought to duplicate upon these animals merely those tonic reactions which one ordinarily experiences in baths of 32 to 34°C. In illustration of the results I insert in this place a table giving the values of the carotid bloodflow and of the arterial pressure as determined by experiment 2 of this series. The immersion was continued in this case during a period of about ten minutes. The water was cooled to 28°C. A comparison of the values before and after the immersion shows very clearly that the cool water exerts a favorable influence upon the bloodflow and that the increased flow is associated with a well marked rise in the systemic pressure and a slight acceleration of the respiratory movements.

[1] The recording stromuhr described by Burton-Opitz was employed (Pflüger's Arch., 1908, cxxi, 150).

TABLE 1

Carotid bloodflow during bath in cool water (Dog 9.5 kilos)

NO. OF PHASE	QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD-FLOW	PRESSURE	PROCEDURE
	cc.	*seconds*	*cc./sec.*	*mm. Hg.*	
1	18.8	12.0	1.57	106.7	Normal
2	18.5	11.0	1.69		
3	19.0	13.3	1.43		
4	19.0	12.4	1.52		
5	18.4	10.8	1.71		
6	18.8	11.4	1.64		
7	19.2	12.8	1.51		
8	19.2	11.3	1.70		
9	19.6	13.1	1.49		
10	19.0	11.3	1.67		
11	19.4	11.5	1.69		
12	18.8	12.2	1.54		
Average.....			1.59	106.7	
13	19.0	11.5	1.65	110.5	Immersion in water of 28°C., 10 minutes.
14	19.0	10.2	1.86		
15	18.2	9.7	1.87	114.3	
16	18.2	9.0	2.02	118.3	
17	18.8	9.8	1.91		
18	18.8	10.4	1.71		
19	19.2	9.5	2.02		
20	19.4	10.2	1.90		
21	19.4	9.4	2.06		
22	18.8	10.6	1.77		
23	19.4	10.8	1.80	119.5	
24	19.0	10.0	1.90		
25	19.2	10.4	1.84		
26	18.8	10.8	1.74		
Average.....			1.86	119.5	

II. THE DISTRIBUTION OF THE BLOOD DURING STIMULATION OF THE SPLANCHNIC NERVE

The quantitative measurements of the bloodflow in the portal vein and its tributaries (1) have proven conclusively that vasoconstrictor reactions in the viscera innervated by the splanchnic nerve caused a marked diminution in the influx of arterial blood into these organs. This stagnation is responsible for the increase in the arterial pressure.

Edwards (2) has shown, however, that the stagnation is not absolute and that a certain compensation is possible through the circuit of the carotid and femoral arteries, which tends to keep the venous system supplied with almost normal quantities of blood.

TABLE 2

Flow in inferior vena cava during stimulation of greater splanchnic nerve

NO. OF PHASE	QUANTITY OF BLOOD	DURATION OF PHASE	BLOOD-FLOW	PRESSURE (MM. Hg.)		PROCEDURE
				Vena cava	Femoral artery	
	cc.	*seconds*	*cc./sec.*			
21	20.0	4.0	5.0	1.5	91.4	Normal
22	20.0	3.4	5.9			
23	19.5	3.6	5.4			
24	19.5	3.5	5.5		91.0	
Average.....			5.4	1.5	91.0	
25	19.8	4.1	4.8	1.5		Stimulation left great splanchnic nerve, 40 seconds, 15 cm.
26	19.8	3.6	5.5		111.5	
27	19.9	3.8	5.2			
28	20.0	4.0	5.0		127.8	
29	20.0	2.8	7.1			
30	20.0	3.1	6.4	0.8	132.4	
31	20.0	2.1	9.0			
32	20.0	2.4	8.3		132.0	
33	20.0	3.0	6.6			
34	20.0	2.0	10.0		132.5	
35	19.8	2.9	6.8			
36	19.8	2.0	9.9	2.0	132.0	
37	19.6	2.5	7.8			
38	19.4	2.0	9.7		128.9	
39	19.4	2.1	9.2			
40	19.3	2.0	9.6		125.8	
41	20.0	2.8	7.1			
42	20.0	2.2	9.0		118.4	
43	20.0	2.6	7.6			
44	20.0	1.8	11.0		114.4	
45	19.4	2.4	8.0			
46	19.4	2.0	9.7	4.0	108.7	
47	20.0	2.4	8.3			

This compensation in the distribution of the blood in consequence of splanchnic constriction is especially well betrayed in the inferior cava distally to the entrance of the hepatic veins. I have succeeded in showing this by calibrating the bloodstream in this vein before and

Fig. 1. Flow in inferior vena cava during stimulation of greater splanchnic nerve (Red. to ⅔ original).

during the excitaton of the left greater splanchnic nerve. The stromuhr was inserted distally to the left renal vein. The distal cannula was connected with a membrane manometer which registered the venous pressure. The general arterial pressure was recorded by a mercury manometer connected with the left femoral artery. In illustration of the results I insert at this time the calculations for a part of experiment 2 of this series, as well as a reproduction of the phases recorded by the stromuhr during this time. As is clearly shown in figure 1, the stimulation of the aforesaid nerve *A–B*, is followed by a decided increase in the venous return, the normal flow of 5.4 cc. in a second, *St*, being gradually displaced by a flow equalling 9.0 to 11.0 cc. in a second. This change occurs during the period of high arterial pressure, *C–D*, i.e., at a time when the splanchnic organs are constricted. Under ordinary conditions the results of this constriction betray themselves in the central venous channels by a drop in pressure in consequence of the lessened influx of portal and renal blood, *F*. The present experiments, however, show that this central deficiency in blood is soon compensated for by a greater influx through circuits not dominated by the splanchnic nerve. In addition this change in the distribution of the blood acts as a check upon the pressures, preventing the occurrence of an undue arterial stagnation and hence of an injurious increase in the arterial pressure.

III. THE RELATION BETWEEN THE INTRAPERICARDIAL PRESSURE AND THE PORTAL BLOODFLOW

These experiments are intended to illustrate the clinical picture observed in pericarditis as it betrays itself in changes in the dynamical conditions of the portal system. They were performed upon dogs during ether narcosis. The chest having been opened and artificial respiration instituted, a glass cannula was inserted in the pericardial sac. The stromuhr was then connected with the portal vein. The venous pressure was registered by a membrane manometer connected with the distal cannula of this instrument, and the arterial pressure by a mercury manometer connected with the carotid artery. The procedure consisted in all cases in obtaining these different records under normal conditions as well as during periods of increased intrapericardial pressure. The latter end was attained by permitting air from a pressure-bottle to flow into the pericardial sac until a very moderate degree of inflation had been established. The height of this pressure was recorded by a water-manometer.

As the results are perfectly uniform, a brief discussion of figure 2 will no doubt suffice to show their character. In the present animal, a dog weighing 14 kilos, the flow in the portal vein, *S*, amounted to 3.69 cc. in a second. The inflation of the pericardial sac was begun at *A*. It was continued during a period of about twenty seconds, i.e., to point *B*, reaching a maximal value of 4 mm. Hg. It is evident in the record that the flow decreases very markedly at *A* and continues small for some time after the cessation of the inflation, i.e., to about point *C*. The average flow during this period is only 1.81 cc. in a second, a reduction of 50 per cent.

Fig. 2. Portal bloodflow on increasing intrapericardial pressure. *A* to *B* (Red. to ⅓ original).

If we now observe the records of the pressures, it is apparent that this period of decreased flow coincides with a fall in the arterial pressure, *CA*, and a rise in the venous pressure, *PV*. These phenomena of low arterial driving force and venous stagnation disappear soon after the cessation of the inflation. It need scarcely be mentioned that greater increases in pressure do not alter the character of these changes but merely tend to render them more conspicuous.

IV. THE INFLUENCE OF TRICUSPID REGURGITATION UPON THE PORTAL BLOODFLOW

The method employed to reproduce the clinical picture of tricuspid regurgitation as it betrays itself in changes in the portal system was

very similar to the one just described. The stromuhr was inserted in the portal vein centrally to the splenic vein. The arterial and venous pressures were recorded by a mercury and membrane manometer connected with the femoral artery and portal vein respectively. The procedure consisted in rendering the tricuspid valve incompetent at a certain moment after the beginning of the calibration of the normal blood flow. The latter end was attained by severing some of the chordae tendineae with a long hook-shaped knife inserted through the right external jugular vein. The chest remained closed.

This procedure was followed by an almost immediate reduction in the portal bloodflow and a fall in the general arterial pressure. The conspicuousness of these changes varied with the severity of the lesion, a gradual cessation of the flow following an absolute incompetency of this valve. In these cases the portal pressure also showed a decrease in accordance with the fall in arterial pressure. Milder types of regurgitation, however, were associated with a slight rise in venous pressure. The reversion appeared when the blood flow had suffered a reduction to about four-fifths of normal.

V. THE BLOOD SUPPLY AND INNERVATION OF THE GALL BLADDER

The attempts which I have made to determine the blood supply of the gall bladder of dogs have not been wholly successful, because the artery supplying this organ is small and rather inaccessible. To begin with, I sought to overcome this difficulty by restricting the vascular field to be experimented upon by ligating the branches of the hepatic artery, so that the main trunk of this blood vessel could be made accessible to the recording instrument. In studying the distribution of this artery with the help of paraffine injections it was found, however, that its branches are distributed not only to the gall bladder but also to the neighboring mass of the liver. Moreover, as it is practically impossible to isolate the latter under the conditions made necessary by experiments of this kind, the results of these calibrations leave much to be desired and shall for this reason not be discussed further.

There is one observation, however, to which I should like to call attention and that is the musculo-motor effect which the stimulation of the coeliac and hepatic plexuses produces. Obviously, as the contraction of the musculature of this organ tends to vary the flow through the distal blood vessels, the flow in the main channel must suffer a similar disturbance. For this reason quantitative fluctuations frequently re-

sult which can scarcely be differentiated from those having a true vasomotor origin.

The contractions of the gall bladder were registered in these experiments by a very sensitive tambour connected with the cystic duct directly below its point of origin. The hepatic plexus was then divided at a point about 2 cm. distally to the coeliac ganglion. Its distal end was placed in shielded electrodes. In another animal, the electrodes were applied to the fibers which eventually escape from the hepatic plexus to form a network around the second branch of the hepatic artery in the immediate vicinity of the common duct.

Fig. 3, Contraction of gall bladder on stimulation of coeliac plexus (Red. to ⅔ original).

In either case the stimulations resulted in well-marked contractions of the gall bladder, proving thereby that the musculo-motor fibers of this organ are derived from the coeliac ganglion and its distal plexus. The accompanying figure (3) shows the record of the tambour *A*, in relation with the blood pressure in the femoral artery, *P*. The latter presents no variations because the hepatic artery had been obstructed in this case some time before the stimulation. At other times, however, when this artery had not been compressed, the stimulations led to a decided increase in the arterial pressure, obviously because the hepatic plexus embraces constrictor fibers for the blood vessels of the liver, the excitation of which lessens the normal arterial flow into this organ (3). The

smaller oscillations upon line *A* are caused by the movements of the diaphragm. The pressures developed by the gall bladder during these stimulations did not exceed 3.0 mm. Hg.

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THE REACTION OF THE KIDNEY COLLOIDS AND ITS BEARING ON RENAL FUNCTION

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The factors underlying the accumulation of dissolved substances in the body fluids, or their secretion, can best be studied by considering three phases in the history of the dissolved substance, namely, the origin or source of supply, the mechanism of distribution and the destination, whether storage, excretion or conversion into other substances. In all of these stages we deal with relative solubilities. Under one set of conditions substances appear to be more soluble in the blood and under other conditions in the relatively more soluble tissue constituents. At present there are two descriptions of the phenomenon of relative solubility which take into account its variations in direction and amount of action. Some emphasize the conditions which point to osmosis as an important mechanism, whereas others see in the colloidal nature of the tissue structure the clue to their behavior. Can the behavior of the tissues and organs be described both quantitatively and qualitatively on a basis of colloid behavior? If so, have we any mechanism which is adequate to accomplish the observed results, in affecting the colloids?

It is well known that many substances in the colloid state can be made to absorb or give up water under varying acid-alkali (1) or hydrogen-hydroxyl (2) concentration. That these observations held good for certain body proteins was emphasized by Martin Fischer in his application of this principle in the study of oedema (1). The question of whether this relationship of ability to hold or release, under varying degrees of "acidity,' held true for dissolved substances was the next point to be solved. Fischer found that fibrin would hold or release sodium chloride, according to whether the acid content was increased or decreased. Later Reemelin (3) showed the same to be true for fibrin and phenolsulphonephthalein. In testing out other substances and

other colloids, Reemelin and Isaacs (2) found that this same relationship held true for the kidney colloids and urea, and that the kidney colloids could be made to secrete more water and more urea, the lower the hydrogen ion concentration, and less when the hydrogen ion concentration was higher. As this extremely delicate relationship was demonstrated to be true for solutions whose strengh and hydrogen ion concentration were within physiological limits, the oft repeated criticism that Fischer's tenets held only for amounts of acid never met with in the body, was answered. This led to the formulation of a theory of *selective adsorption*, which held that a dissolved substance could be held or passed on, according to its behavior in the presence of a higher or lower hydrogen ion concentration. It thus became desirable to test out other substances and note their behavior to the kidney colloids. In this way one of the factors involved in the accumulation or decrease of dissolved substances in the body fluids could be studied. The present paper deals with the relationship between sodium chloride and the kidney colloids, under varying degrees of hydrogen ion concentration and varying strength of salt solutions. The material also allowed the taking of some observations on the excretion of albumin and its relation to the secretion of water and salts.

MATERIAL AND METHODS

In the following experiments kidneys of rabbits, under ether or chloroform anaesthesia, were isolated and an artificial circulation was set up through cannulas inserted in the arteries and veins. The "urine" was collected from cannulas tied in the ureters. The kidneys in each case were removed from the body and kept moist. The perfusion was carried out by the method described by Sollmann (4) and used by Reemelin and Isaacs (2) in the study of urea excretion. The perfusing solution consisted of sodium chloride in distilled water, and the hydrogen ion concentrations were varied by means of traces of phosphoric acid, disodium hydrogen phosphate or sodium hydroxide. From 100 to 200 cc. more or less, of each perfusing solution was used before it was replaced by another. In order to facilitate the change of solutions several reservoirs were used, each connected with the same glass tube and controlled by pinch clamps, so that a solution could be turned on or off and be replaced by a new one without danger of disturbing the kidney or admitting air bubbles. The blood pressure was kept constant throughout the experiments. The perfusing solutions were of 0.95 per

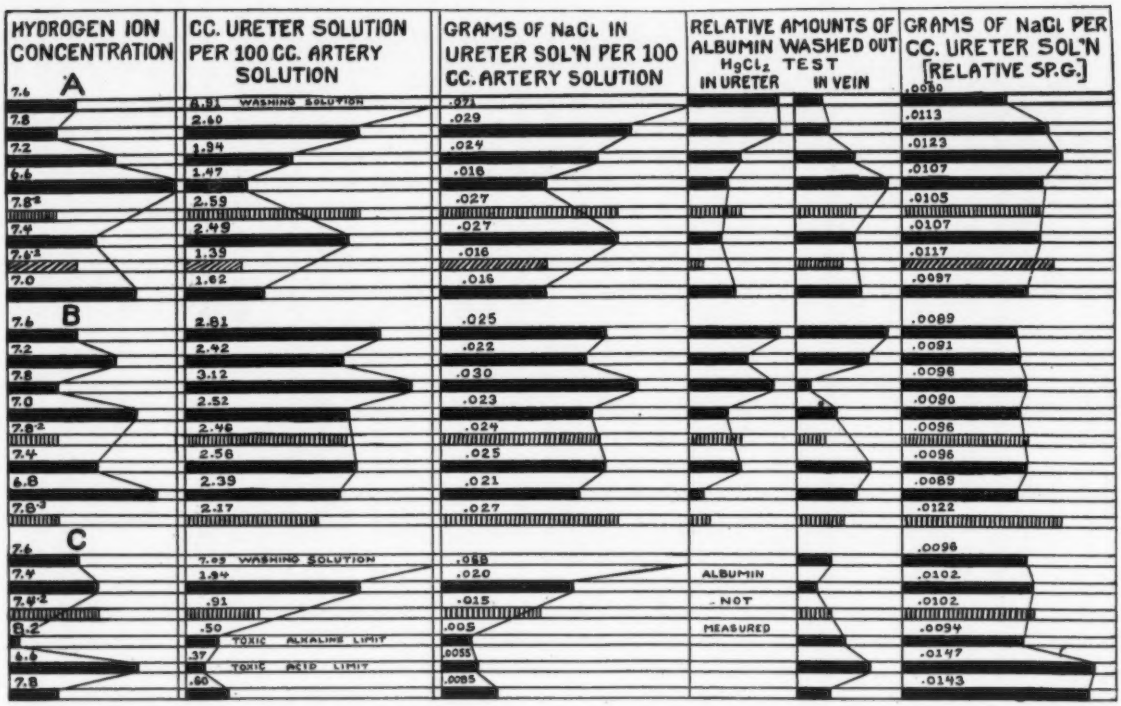

Fig. 1. Solutions are listed in the order of their perfusion. Each experiment represents a separate kidney. To read chart easily, hold page so that blackened lines are vertical.

cent, 0.88 per cent and 0.87 per cent of sodium chloride. By means of a stream of air bubbling through the solution in the reservoir, the oxygen tension was kept constant. The hydrogen ion concentration varied from pH–6.6, the most acid used, to pH–8.2, the most alkaline. The blood was washed out of the vessels with a salt solution of pH–7.6.

The hydrogen ion concentration of the artery was always retested from samples taken from a valve near the kidney, so that any change in the reaction, brought about by contact with the rubber and glass tubes leading from the reservoirs, could be detected. The hydrogen ion concentrations were determined by comparisons of measured amounts with solutions of known strength, using phenolsulphonephthalein as the indicator. The solutions were collected from the vein and ureter separately, the amounts measured and the amount of the chlorides determined by Vollhard's method. The details are given in the accompanying table.

The table and figures show the order in which the solutions were perfused, the hydrogen ion concentrations and the amount of salt and water excreted. It is to be noted that during any one experiment the chloride content of the artery solution was constant, and proteins and substances in the colloid state were absent. The solutions differed from one another in the presence of the smallest possible traces of phosphoric acid, disodium hydrogen phosphate or sodium hydroxide that would give the proper hydrogen ion concentration. This amounted to a fraction of a milligram in many cases, amounts too small to account for the results purely on a basis of osmosis. The results show that *the secretion of both sodium chloride and of water drops as the solution becomes more acid or less alkaline, within physiological limits, and rises when the order is reversed.* That this is true regardless of the order of perfusion is shown in the experiments which were arranged so that sometimes the variations were from acid to less acid or alkaline, and sometimes in the opposite direction.

A suggestive fact to be noted from these experiments is that when a solution at a given hydrogen ion concentration is repeated, that is, after about 100 cc. have been perfused, if a second perfusion of like amount is tried, the results are below normal. This is confirmed by other experiments and probably correlated with the fact that certain albumins, whose presence were shown by reagents, are more soluble in the sodium chloride solution at one hydrogen ion concentration than at another, and leave the kidney by way of the vein or the ureter. With the removal of these albumins the ability of the kidney colloids

to secrete sodium chloride and water is lessened inasmuch as no repair can take place in the kidney under the conditions of the experiment, blood being absent. In experiment B, the solution pH–7.8 was perfused three separate times, the amount of albumin per cubic centimeter as well as the total amount of albumin in the vein increased, but that in the ureter decreased. The approximate measurements of the relative total amount of albumin present in each ureter solution showed that the amount of albumin increased with the decrease in hydrogen ion concentration, and decreased as the hydrogen ion concentration increased. In this respect it followed the secretion of salt and water. This result does not at first appear to bear out some clinical correlations because of a common error sometimes used in reporting the amount of albumin in the urine, as well as the amount of sodium chloride or urea in a specimen. It is of course evident that any quantitative reading which expresses the concentration of a substance gives no clue as to the entire amount of that substance present. One commonly sees tables in the journals giving grams of urea, sodium chloride or other substance per 100 cc. of a fluid and comparisons made on this basis. Clinically it is quite common to see the direct reading of the Esbach albuminometer on one occasion compared with the corresponding reading on another occasion, and the conclusion of improvement or turn for the worse based on this. It is evident that the only factors that can be compared are the total or relative amounts of substance present in a given period of time or of equivalent function. These experiments show that, dependent upon the total amount of water excreted, the total amount of albumin lost by the kidney (and therefore, as the quantitative data show, corresponding decrease in function), may be more or less than when a different amount is excreted although the concentration in grams per unit volume may be the same. When these facts are taken into account it is seen that when a small quantity of liquid washes out as much or more albumin than a larger quantity in a given time, it is to be expected that the disturbing cause is more marked or widespread in the first than in the second. These results during life would be modified by two factors, the repair of the kidney and the blood as a source of albumin.

The relationship of albumin to water excretion, however, is not as simple as merely a matter of mechanical solution and washing action. There is some evidence to indicate that solutions of different hydrogen ion concentrations washed out different kinds of protein in different amounts. The amounts of protein were measured in several ways.

Turbidity of columns having a proportionate relative length were compared, amount and volume of precipitate was noted as well as total amount of reagent necessary to produce maximum turbidity. Various reagents, as nitric acid, mercuric chloride, picric acid, potassium ferrocyanide, acetic acid, as well as heat, and combinations of these, gave different amounts of protein for the same solution, so that the order of concentration varied when arranged on the basis of behavior toward any given reagent. The difference was more than could be accounted for on a basis of acid albumin and alkaline albumin. Furthermore the precipitate of albumin settled to the bottom of the comparison tubes at different rates, which were not coördinate with the order of concentrations as shown by the precipitating reagent. This, together with the microscopic evidence, details of which are given below, suggests that we are dealing with solutions whose composition differed in kinds of protein present as well as in amounts.

The precipitated albumin when examined under the microscope showed characteristic precipitation patterns, which differed in complexity and structure. The microscopic picture resembled very much in form and staining qualities the fibrillar networks often seen inside the kidney cells and between them, in histological sections of "fixed" kidney tissue. This method of studying the meaning and composition of some of the structures seen in fixed tissue is now being followed and the significance of these appearances will be discussed in a later paper (see also Isaacs, (5)).

It will be noted that alkaline solutions as well as acid, washed out albumin. As we often note that clinically an alkaline urine may contain albumin just as well as an acid specimen, it is possible that the points noted on the kidney colloids in these experiments may have some relation to the condition in some living kidneys. Thus far, substances responding to the tests for serum albumin, nucleoprotein, mucin, histone and some globulins have been identified. The observations taken so far are not extensive enough to permit the publication at this time of a series showing the quantitative relation between the kind of albumin and the hydrogen ion concentration. These differences suggest that clinically the kind of albumin which predominates in the urine may be a clue to the nature of the toxic agent causing its excretion. It will be noted from the chart that as a solution at a given hydrogen ion concentration is repeated, there is a tendency for more albumin to be washed out in the vein and less in the ureter. If such a condition were present during life, one would expect a variation in the kind and amounts of albumin in the blood.

The decrease in the excretion of sodium chloride and of water, when a solution at a given hydrogen ion concentration is repeated, and the recovery, as far as other solutions are concerned, suggests that there may be *a definite relation between the kind of albumin washed out in the ureter and the hydrogen ion concentration of the perfusing solution.* This is compatible with the idea of a selective adsorption (2) as far as both sodium chloride and proteins are concerned on a basis of variation in affinity with variations in hydrogen ion concentration. In similar experiments (fig. 2) the secretion of the repeatedly perfused kidney was restored, quantitatively, to normal when defibrinat d blood was added to the perfusing solution, the excreted protein being thus replaced.

The lessened effect of the repeated solution amounts in the end to a form of tolerance, which suggests a possible mechanism for drug tolerance noted clinically. The selective secretion of "Bence-Jones protein" described by Taylor, Miller and Sweet (6) may be a form of excretion similar to that noted in these perfusion experiments. The relationship of increased excretion (from the point of view of normal function) with decrease in hydrogen ion concentration is probably limited, as far as the kidney colloids are concerned, to hydrogen ion values which are within the narrow physiological limits. In these, as in other experiments, an "acid" concentration as high as pH–6.6 ($\frac{1}{3,981,000}$ gram of hydrogen ions per liter) proved toxic by reducing the secretion, whereas the alkaline pH–8.2 ($\frac{1}{158,500,000}$ gram of hydrogen ions per liter) had a similar effect, although the alkaline upper limit is probably higher than this. The secretion curve reaches its highest point between pH–7.2 and pH–7.8.

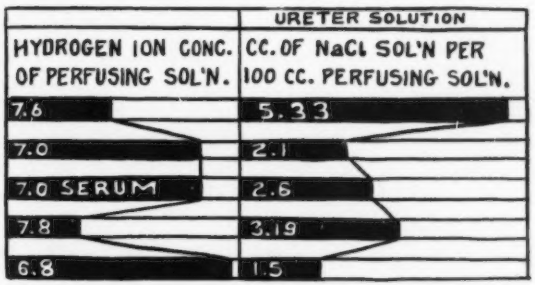

Fig. 2. Experiment D. The perfusion of a kidney with serum restores the function by replacing albumin washed out.

The biological effects of neutral salts in reducing the affinity of acidified hydrophilic colloids for water has been noted by Fischer. As it is in the swelling or reduction in size that Fischer sees the mechanism of adsorption and secretion, it is then of interest to see if neutral salts

affect the secreting mechanism in these experiments. That there is an effect, these experiments illustrate very well. In experiment B the average of the hydrogen ion concentrations was pH–7.4 which is the same as that in experiment C. However, in experiment C 100 cc. of artery solution produced only 1.367 cc. of ureter solution as compared with 2.574 cc. of ureter solution for the same amount of artery solution in experiment B. In experiment C the smaller amount of secretion was correlated with a higher concentration of sodium chloride in the artery solution, namely 0.95 per cent as compared with 0.88 per cent in experiment B. A possible explanation of the lessened secretion in experiment C may be a force analogous to that which causes neutral salts to decrease quantitatively the swelling of colloids, as taught by Fischer. In these experiments it was not possible to take quantitative data to show whether secretion changes were accompanied by swelling changes, because of mechanical difficulties. That solution of protein took place has been noted, and it is known that in many colloids the solution process runs a course somewhat parallel to the swelling process, often accompanying it. Certainly the "kidneys" after the experiments were completed showed a typical picture of "cloudy swelling," but the change in size due to the blood pressure was such that minute changes in the size of colloids or their change back and forth from a precipitated form to a soluble form could not be measured under the conditions of the experiments. Furthermore, the process of cloudy swelling, with all the changes that go with it, evidently produces a change of elasticity in the kidney so that the same blood pressure will cause the kidney volume to increase more in the later stages than in the earlier (7).

The concentrations of the ureter solutions taken in grams of sodium chloride per cubic centimeter (see fig. 1) indicate that the secretion of sodium chloride is probably quantitatively not dependent on the secretion of water as, in the presence of a constant amount in the artery solution, the concentrations of some ureter solutions increased with the decrease in hydrogen ion concentration, whereas in others it decreased. In experiment C there is a consistent variation of the concentration of sodium chloride with that of the hydrogen ions, both increasing and decreasing together. This direct variation held true for only some of the other perfusions. In two cases (pH–7.4 in experiment C and pH–7.8 in experiment B) the repetition of a solution gave ureter solutions of practically the same concentration (number of grams of sodium chloride per cubic centimeter) but the relative amounts

excreted (amount of sodium chloride per 100 cc. of artery solution) were different, as pointed out before. If these results apply to the living kidney one would not expect to correlate the concentration of the urine directly with its acidity or with the hydrogen ion concentration of the blood, as the chart clearly shows. A third factor, namely, the colloid most affected at the hydrogen ion concentration in question, evidently must be taken into consideration.

The experiments on urea and on sodium chloride have shown that in the presence of a constant concentration of these substances in the artery solution, the amount secreted varied and the variation was closely related to the hydrogen ion concentration of the artery solution. They have also shown that although a chemical analysis may show the presence of a certain amount of urea or sodium chloride in the blood, yet the amount *available* for secretion may not be the same. Thus although an artery solution contained a constant amount of an aqueous salt solution, a variation of over 36 per cent excretion was noted in one experiment, the only variable factor being a change in hydrogen ion concentration from pH–7.2 to pH–7.8. While secretion of both urea and sodium chloride increases as the hydrogen ion concentration decreases, this relation does not hold true for all the colloids in the body. Fischer (1) as stated before, finds that fibrin will hold sodium chloride, the more acid it is, while Reemelin finds that this does not hold for fibrin and urea. On this basis we can expect diminution in the chloride secretion ("salt or chloride retention") in the presence of an apparently good urea output, as, of the blood and kidney colloids, both tend to hold the chlorides, while of the two, only the kidney colloids tend to hold the urea. This condition is noted clinically in some conditions of chloride retention. Wolferth (8) notes that chloride retention in eclampsia may be marked while the retention of urea may be very slight.

The results of experiments on glucose, creatin, creatinine, uric acid and phenolsulphonephthalein will be reported later.

SUMMARY AND CONCLUSION

1. In isolated rabbit kidneys, perfused with salt solutions of constant and known composition, the secretion of sodium chloride and of water increases with the decrease in hydrogen ion concentration and decreases as the hydrogen ion concentration increases.

2. This relationship holds true for hydrogen ion concentrations within physiological limits.

3. pH–6.6 probably represents the acid limit for function while the alkaline limit is pH–8.2 or higher. Variations beyond these are highly toxic to the kidney colloids and reduce secretion. The optimum hydrogen ion concentration lies between pH–7.2 and pH–7.8.

4. The amount of sodium chloride found in the blood on chemical analysis does not indicate the amount available for secretion. The amount available varies with the hydrogen ion concentration.

5. The effect of neutral salts (sodium chloride) in preventing the kidney colloids from taking up water and salt and secreting it can be shown quantitatively by varying the strength of the perfusing solution.

6. That the kidney colloids can be the source of albumin in the urine is shown by the fact that an artificial albuminuria, responding to the clinical tests, can be produced even though the artery solution contains no protein.

7. The amount of albumin washed out varies with the hydrogen ion concentration and follows the secretion of salt and water in which, under the conditions of the experiment, it becomes soluble.

8. There is evidence that some of the kidney proteins are more soluble in solutions of one hydrogen ion concentration than in another. This may account for the variations in kind of "albumin" found clinically in urine analyses, as well as the selective secretion of proteins.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XLIV. The Origin of the Epigastric Pains in Cases of Gastric and Duodenal Ulcer

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The genesis of the pains of gastric and duodenal ulcers is not yet satisfactorily demonstrated. The view that *the pains are due to acid irritation of hyperexcitable nerve endings or exposed nerve fibers in the ulcer area* has probably the greatest number of adherents at present. The essential facts in support of this view are: 1. Analogy. Acid irritation of abraded areas of the skin, mouth or nose epithelium, etc. produces pain. 2. The frequent occurrence of so-called gastric hyperacidity in gastric and duodenal ulcers. 3. The temporary alleviation of the ulcer pain by food and alkalies.

The following facts appear to run counter to, or are at least not readily explained by this acid corrosion theory:

1. Gastric ulcer with or without clinical hyperacidity[1] may be present without pain.

2. Gastric ulcer and ulcer pain may be associated with normal acidity, and even with hypoacidity.

3. The pains of gastric ulcer may be present and be temporarily relieved by food or alkalies, even though the stomach contents are alkaline (1).

4. Introducing acids (0.5 per cent HCl) into the stomach does not, or at least not invariably, induce or augment the ulcer pains in gastric ulcer patients (7).

[1] While there is no evidence that Alexis St. Martin at any time had acute or chronic ulcer of the stomach or duodenum, Beaumont on several occasions observed raw patches on the gastric mucosa from which blood or pus exuded. In most instances this condition of the mucosa did not cause pain or discomfort, even though the abrasions of the mucosa persisted for several days. Beaumont remarks (p. 240): "It is interesting to observe to what extent the stomach may become diseased without manifesting any external symptoms of such disease."

5. The ulcer pains usually show a periodicity (being described as "gnawing" or "boring"), and the periods are too short to be explained by variations in the gastric acidity.

The other leading view *ascribes the ulcer pains to contractions of the stomach, the pylorus and possibly the upper end of the duodenum, the excessively painful character of these contractions in ulcer cases being due either to hyperexcitability of the gastric pain nerves or to abnormally strong contractions.* This theory has been fortified by clinical observation and experimental data especially by Hertz (7), and striking confirmatory findings on ulcer patients have recently been reported by Ginsburg, Tumpowsky and Hamburger (5). The following facts appear to support this theory:

1. Strong contractions or a certain type of contractions of the stomach and intestines give rise to varying degrees of pain in the absence of ulcer ("hunger pangs," "colic," tenesmus, various forms of gastralgia, etc.).

2. Pain nerves appear to be absent from the gastric mucosa (4).

3. The evident synchrony of the ulcer pains with gastric and possibly pyloric contractions, so far as this phase has been studied by the balloon and X-ray methods (4), (5), (7), (13), (14).

4. The frequent association of typical gastric ulcer pains with appendicitis, cholecystitis and even achylia gastrica, (6) not complicated with gastric ulcer.

5. The similarity of the moderate ulcer pains as regards periodic exacerbation with the strong hunger pangs of normal persons, a similarity that lead Moynihan and others to designate the pains of gastric and duodenal ulcers as "hunger pains."

But while the gradually accumulating reliable data thus point to gastrointestinal contractions as the immediate cause of the ulcer pains, we do not know: (1) what part of the digestive tract (stomach, pylorus or duodenum) is primarily concerned; (2) whether the site of the painful contraction varies with the location of the ulcer; (3) or what is the relation of gastric acidity to the initiation of the contractions. The observations here reported were undertaken in the hope of securing clearer knowledge of these factors.

Mr. G. H. M., age 25, the subject in these studies, was at the time a medical student in our laboratory. Six months before coming under our observation he suffered an acute attack with all the typical symptoms of "peptic ulcer," including hematemesis. After several weeks in a hospital on medical treatment and an ulcer diet, he recovered suf-

ficiently to resume his medical studies.[2] But despite the improvement the ulcer had evidently not healed, as he suffered periodic exacerbations of the ulcer syndrome, even though he continued the ulcer diet fairly consistently. These attacks usually lasted from three to six days and came at irregular intervals, a few days to several weeks apart. Dietary indiscretions or excessive mental work appeared to induce or aggravate these attacks.

During the three months of these observations Mr. M. experienced two of these relapses, one of them being very severe. Between these attacks his gastric motility and gastric sensations were those of a normal person.

1. When the gastric hunger pains are of moderate severity there is practically complete synchrony of the appearance, intensity and duration of the ulcer pains and the stomach contractions (fig. 1)*; and these contractions are not stronger or more prolonged than the stomach contractions felt as ordinary hunger pangs on days when the subject has no ulcer symptoms.*

That the gastric contractions give rise to the ulcer pains, and not *vice versa*, is shown by the fact that the pains are not felt until the contractions are well developed, or frequently not until the fundic part of the stomach has begun to relax or is completely relaxed. That is to say, the gastric contractions precede the ulcer sensation, just as they precede the hunger pang. The reader will recall that in our graphic method of recording the gastric contractions the balloon is lodged in the fundus and body of the stomach. And since the ulcer or hunger contraction sweeps over the entire stomach as groups of peristaltic waves beginning at the cardia (10), it is evident that when the fundus starts to relax the pyloric end of the stomach will be strongly contracted. The fact that Mr. M. in most cases did not begin to feel the ulcer pains until the contractions involved the pyloric end may indicate that it is mainly this region of the stomach, together with the pylorus, that gives rise to the ulcer pains by contraction. If this is a fact it would help to explain the frequent absence of ulcer symptoms when the ulcer is located at the fundic and cardiac regions.

2. The ulcer pains are felt both on the empty and the filled stomach.

[2] February 26 to March 18, 1917, the Nebraska Methodist Hospital, Omaha. At that time Mr. M.'s stomach content and feces showed occult blood. The x-ray examinations revealed gastric hyperperistalsis and rapid emptying. The Roentgenologist, Dr. C. R. Kennedy, made the diagnosis of duodenal ulcer. The internist in charge of the patient, Dr. E. L. Bridges, made the diagnosis of "peptic ulcer," the exact location of the ulcer being uncertain.

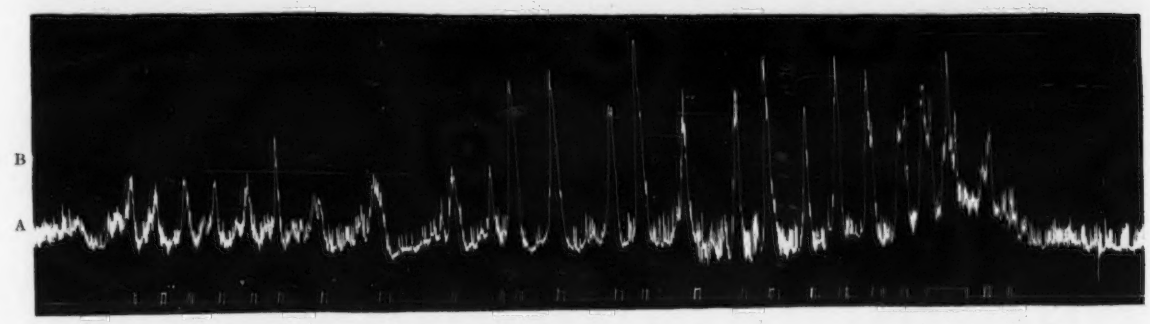

Fig. 1. G. H. M. August 10, 1917. Record of the sensation of gastric ulcer by means of a recording signal, *b*, parallel with the recording of the contractions of the stomach by the balloon method, *a*. Chloroform manometer. Contents of the stomach at the beginning of the record 65 cc., trace of bile, but no food remnants. Free acidity = 0.18 per cent, total acidity = 0.24 per cent. Stomach contents at the end of the record 25 cc. + bile. Free acidity = 0.15 per cent; total acidity = 0.21 per cent.

This record shows that in ulcer patients the contractions of the empty stomach—felt as hunger pangs by normal persons—induce the sensation of gastric ulcer pains.

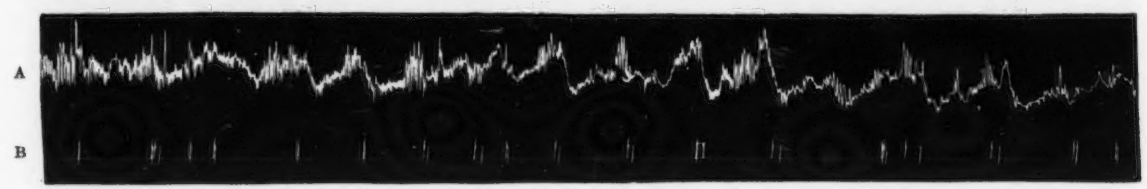

Fig. 2. G. H. M. August 3, 1917. Record of the sensation of gastric ulcer pain by means of a recording signal, *b*, parallel with the recording of gastric contractions by the balloon method, *a*. Mr. M. had a bowl of tomato soup and a glass of milk at 12.30 p.m. He began to feel the ulcer pains distinctly at 1.30 p.m. The graphic observations were made from 3 to 6 p.m. At 3 p.m. 100 cc. of chyme containing tomato débris, some milk curds and a few fat droplets, were removed from the stomach. Free acidity = 0.34 per cent; total acidity = 0.44 per cent. At the end of the observation period 35 cc. of nearly clear fluid was removed from the stomach. Free acidity = 0.34 per cent; total acidity = 0.41 per cent.

This tracing shows that in gastric ulcer patients the tonus rhythm of the fundus associated with the normal digestion peristalsis is felt as ulcer pains.

The ulcer pains of moderate severity felt on the partly filled stomach are caused by a gastric tonus rhythm apparently identical with that seen in the normal digestion peristalsis (fig. 2).

The degree of tonus rhythm of the partly filled stomach felt as ulcer pains by Mr. M. gave rise to no sensations at all during the time of well-being between the two periods of attack. This is typically normal. We have studied the gastric fundus rhythm of normal digestion peristalsis in ourselves and a number of other normal persons. These contractions produce no effects on consciousness, except toward the very end of gastric digestion when they gradually develop into hunger pangs.

The reader will note in figure 2 that the ulcer pains, as a rule, are felt toward or at the end of each tonus contraction, a fact which again points to the antrum and the pylorus as the main factors in the genesis of the ulcer pains, at least in Mr. M. But it should not be forgotten that the relatively feeble or normal digestion contractions of the fundus may be no index of the intensity of the antrum and pylorus contractions.

3. Gastric acidity bears no direct relation to the onset or the severity of the gastric ulcer pains (figs. 3 and 4). This appears to be conclusively established by the following facts:

1. On certain days of the two periods of relapse Mr. M. was disturbed by ulcer pains only at certain times of the day. On recording the gastric acidity and motility it was always found that when he was free from the ulcer pains the stomach was relatively atonic and quiescent; when the ulcer pains were present gastric contractions were also present (fig. 3). The acidity of the gastric content remained the same, or it might be either lower or higher during the pain period.

2. The same fact is brought out by comparing the gastric acidity on days free from ulcer pains with that on days of marked pain (fig. 4). Thus on the morning of July 28 (no breakfast) the stomach content was 90 cc. with an acidity of 0.20 per cent free and 0.29 per cent total. The balloon showed the stomach quiescent and Mr. M. felt no gastric distress. On the morning of July 31 (no breakfast) the gastric content was 85 cc., free acidity 0.20 per cent, total 0.27 per cent. The stomach showed vigorous contractions and Mr. M. felt the ulcer pains as very severe.

3. On August 8 Mr. M. had breakfast of oatmeal and cream at 8 a.m. The ulcer pains appeared about 9 a.m. and continued all morning. At 12 o'clock noon the stomach contents were 120 cc. of chyme with an acidity of 0.34 per cent free and 0.43 per cent total. At 1 p.m. he ate

two soft boiled eggs, one slice of toast and a glass of milk. The ulcer pains appeared at 2 o'clock and continued throughout the afternoon. At 5 p.m. 150 cc. of chyme were taken out of the stomach, having an acidity of 0.33 per cent free and 0.47 per cent total. On this day Mr. M. evidently showed clinical hyperacidity with some gastric retention.

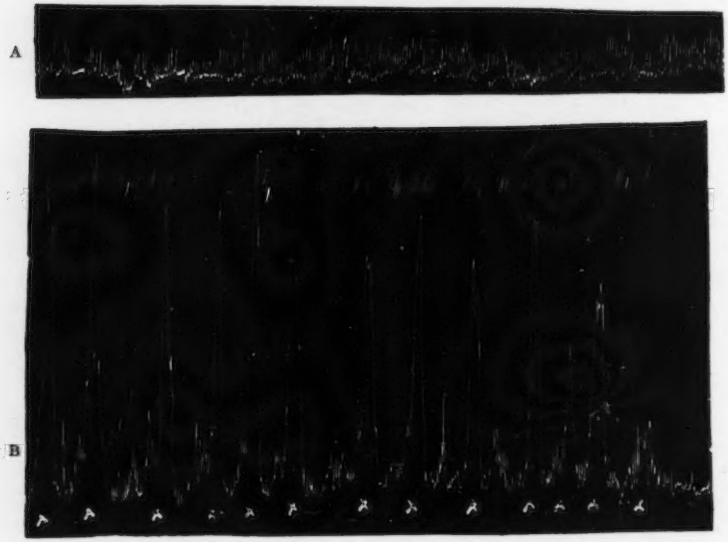

Fig. 3. G. H. M. July 24, 1917. Record of the stomach contractions by means of the balloon method. No breakfast. Stomach contents at 9 a.m., 95 cc.+ trace of bile, no food remnants; free acidity = 0.20 per cent; total acidity = 0.28 per cent. Tracing A taken 9.30 a.m. Stomach atonic and quiescent. Mr. M. felt no ulcer pains. The acidity of the stomach contents was: free = 0.20 per cent; total = 0.27 per cent. Tracing B taken at 11.30 a.m. The time when the ulcer pains are felt is indicated by *X*. The acidity of the stomach contents was: free = 0.18 per cent, total = 0.26 per cent. These tracings show that the ulcer pains are correlated with gastric motility, not with gastric acidity.

But contrast this record with the observations on August 14. On the evening of August 13 Mr. M. had dinner (two soft boiled eggs, two slices of toast and a glass of milk) at 6 p.m. The ulcer pains appeared at 7.30 p.m. They were relieved for a time by taking a glass of water. At 9 p.m. the pains were eased for thirty minutes by taking soda; at 11.30 the pains were relieved by taking two glasses of milk. At 2 a.m.

the ulcer pains woke him up from his sleep and he took 2 gm. soda without relief. At 2.30 he took two glasses of milk, the pains eased up, but he woke up at 5 a.m. with severe pains. Temporary relief obtained from two glasses of water. More water was taken at 7 a.m. No breakfast. At 8 a.m. the stomach content was 120 cc. + trace of bile with an acidity of 0.04 per cent free and 0.10 per cent total. A glass of water was taken at 10 a.m., the ulcer pains persisted throughout the morning. At 1.30 p.m. the stomach content was 65 cc. + bile and mucus with an acidity of 0.03 per cent free and 0.07 per cent total. There was no remission in the ulcer pains. Mr. M. stated that so far as he could make comparisons the ulcer pains on August 14 (stomach empty and actual hypoacidity) were just as severe as on August 8 (gastric food retention and clinical hyperacidity).

4. There is some evidence that the contractions of the pylorus and the upper part of the duodenum may in certain cases contribute to the ulcer pains.

In tracing B, figure 4, the reader will note that Mr. M. records the feeling of ulcer pains, *x*, in some cases when there is no evidence of contractions in the stomach itself. What is the cause of these pains? We believe they are due to contractions of the pylorus and the upper end of the duodenum, on the following grounds:

1. Observations by Dr. A. B. Luckhardt in collaboration with the author have brought out a new fact in the control of the pylorus, namely, a correlation of the opening of the pyloric sphincter and the tonus rhythm of the stomach. *In normal men and dogs the pyloric sphincter opens not with each peristaltic wave but at the end or at the height of each tonus contraction of the stomach.* We do not yet know to what extent this normal mechanism is modified by gastrointestinal disorders or by experimental changes of gastric acidity. It is evident, however, that the mechanism fails to work regularly in cases of pyloric spasms. But pyloric spasms are not an invariable concomitant of gastric and duodenal ulcer.

2. Again, referring to tracing B, figure 4, we call attention to the fact that in many cases at least the moderately severe ulcer pains are felt toward the end or at the very end of a gastrotonic contraction, that is, at the time of the opening of the pylorus and its immediate closure by the acid reflex from the duodenum.

3. It is a fact well known to physiologists that acids or chyme with an acidity of 0.3 per cent to 0.5 per cent acting in the upper end of the duodenum induce not only a reflex closure of the pylorus but a strong

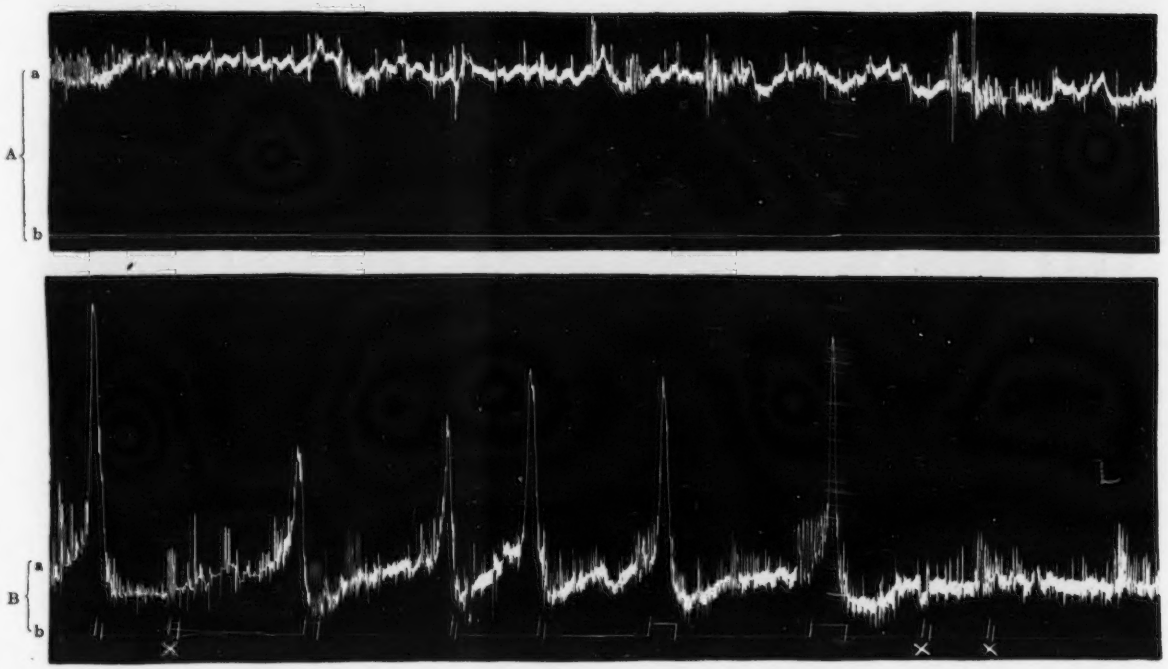

Fig. 4. G. H. M. Record of the sensation of gastric ulcer pains by means of a recording signal, *b*, parallel with the recording of the gastric contractions by the balloon method. Chloroform manometer. Tracing A, July 28, 1917, 11.30 to 12.30. No breakfast; a glass of water at 8 a.m. 11.30 a.m., contents of the stomach 90 cc. + bile. Free acidity = 0.20 per cent; total = 0.29 per cent. At 12.30 p.m. contents of stomach: 85 cc. + bile. Free acidity = 0.22 per cent; total = 0.31 per cent; stomach quiescent and no sensations of ulcer pains.

Tracing B, July 31, 10 to 12 a.m. No breakfast, a glass of water at 8 a.m. At 10 a.m. stomach contents = 65 cc. + bile. Free acidity = 0.20 per cent; total = 0.27 per cent. At 12 m. stomach content = 55 cc. + bile. Free acidity = 0.21 per cent; total acidity = 0.29 per cent. These tracings show that the gastric ulcer pains are correlated with gastric and pyloric motility, not with gastric acidity. For explanation of ulcer pains felt at *X*, see text.

tonic or tetanic contraction of this part of the duodenum itself. This is at least true of dogs under light ether anesthesia. The higher the acidity the stronger and the more prolonged both the pyloric closure and the duodenal spasm (8). It is reasonable to infer that weaker acids, 0.1 to 0.2 per cent, will induce these strong contractions in cases where the duodenal and pyloric nervous mechanism is rendered hyperexcitable by ulcer or other pathological states.

4. Pyloric hypertonicity and spasms in infants may or may not be painful, but internists are generally agreed that in adults pyloric spasms are painful in proportion to their intensity and duration. And if the sensory nerves of the pylorus are hyperexcitable, the ordinary pyloric tonus and contractions may be felt as pain, just as is the case with the normal digestion peristalsis of the stomach in gastric ulcer.

DISCUSSION

All the evidence thus points to the fact that the pains of gastric and duodenal ulcers are contraction pains arising either in the stomach or in the pylorus and upper part of the duodenum. In the case of the stomach the contractions are usually not stronger than those of normal digestion peristalsis of the filled or the hunger tonus rhythm of the empty stomach. This points clearly to a condition of hyperexcitability of the gastric pain nerves in the ulcer patients experiencing the typical ulcer pains.

Since the ulcer pains are due to tension of excessive contractions or of normal contractions on hyperexcitable pain nerves, it is evident that pathological states other than ulcer, inducing such hyperexcitability or hypermotility, will cause symptoms of gastric ulcer pains practically identical with those of ulcer, as appears to be the case in many instances of appendicitis, cholecystitis and achylia.

The contraction origin of the ulcer pains also serves to explain the frequent, if not usual, lack of parallelism between ulcer pains and gastric acidity. Within certain limits the motility of the stomach is independent of the chemical reaction of the stomach contents (3). The influence of the chemical reaction of the gastric content on the pylorus is more complicated (2, 3, 10, 12), but high acidity will intensify and prolong the duodenal reflex contraction of the pylorus as well as induce strong contractions in the duodenum itself. Clinical hyperacidity may in that way indirectly aggravate the ulcer pains from the contraction of these parts of the alimentary canal, and any measure (protein

food, water or alkalies) that temporarily lowers gastric acidity will temporarily ease these pains, provided there is sufficient relaxation of pylorus to allow the weakened gastric content to reach the duodenum.

When the ulcer pains are due to the tonus and contractions of the body of the stomach, any measure which inhibits or decreases the gastric tonus (ingestion of food, water, alkalies, or acids, passing of the stomach tube, etc.) will temporarily ease the pains, irrespective of the chemical reaction of the stomach content.

While it is true that in the gastric ulcer patients, so far carefully studied by the balloon method, the stomach contractions causing the pains have not been stronger than those of healthy persons, there may be cases of ulcer pains actually due to excessive contractions, especially in the case of the pylorus. The continuous epigastric pain at times present in ulcer is probably due to persistent hypertonus of the stomach or the pylorus, and the more severe pains that cause the patient to double up are probably due to pyloric spasm.

The contraction origin of the ulcer pains also helps to account for the many cases of gastric ulcer not accompanied by pain. The variable factors are the gastric, pyloric and duodenal contractions, the proximity of the ulcer to the main branches of the vagi, the extent of inflammation and edema in the ulcer area, and the degree of inherent stability of the autonomic nervous system of the individual. It is obvious that further advance of our knowledge in this difficult field requires a combination of the balloon and the fluoroscope method of objective registration parallel with careful introspection on the part of the intelligent patient.

These newer aspects of the ulcer pains also serve to emphasize the fact that elimination of the ulcer pains is no criterion of healing of the ulcer. This criterion satisfies only the ignorant patient and the careless clinician.

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A NOTE ON SOME OBVIOUS CONSEQUENCES OF THE HIGH RATE OF BLOOD FLOW THROUGH THE ADRENALS

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It has been shown by a number of observers that the rate of blood flow through the adrenal glands is exceptionally great. According to Neuman (1), it amounts in cats to 6 to 7 cc. of blood per minute per gram of organ, with a blood pressure of 130 mm. of mercury, a greater flow than that through any other organ.

Stewart and Rogoff (2) have published results on the rate of filling of a cava pocket from the adrenal veins in cats, from which the rate of flow through the glands with unopened blood vessels and in the absence of hemorrhage can be calculated. For example, in five successive observations while the circulation was fairly good, the blood flows were 2.8 cc., 2.6 cc., 2.2 cc., 2.3 cc., 2.5 cc. per gram of gland per minute. As the mesenteric and coeliac arteries were not tied and the arterial blood pressure was rather low in this experiment (38 to 42 mm. of mercury during the observations quoted), these rates of flow are doubtless below the average with blood pressures more nearly approaching the normal.

In other papers (3), we have published numerous observations in which the blood flow through the adrenals was estimated by collecting the blood through a cannula. In the great majority of these experiments the coeliac, mesenteric and renal arteries and the abdominal aorta were tied. Therefore the arterial pressure at the commencement of collection of the blood was generally high.

In twenty-three cats the blood flows in grams per minute per gram of gland were: 3.3, 4.2, 6.4, 11.3, 5.0, 7.0, 5.6, 4.5, 3.0, 1.4, 2.7, 3.2*, 7.9*, 6.0*, 4.5*, 4.2, 9.1*, 4.9*, 6.6*, 2.0*, 2.5*, 3.1, 6.0. Average, 5.0 grams.

The numbers marked with an asterisk are from animals in which only one adrenal remained, the other having been extirpated some weeks

previously. The average of these results shows practically the same flow as the average of the whole. In all the experiments several samples of blood were collected through the cannula. The rate of blood flow is usually calculated for the second sample, the first being rejected if much higher than the others because of the possibility of some blood being included in the cava pocket at the time it was clipped off, which would of course increase the apparent rate of outflow in the first sample. The results may accordingly be considered as certainly not too high for the given experimental conditions (ligation of alternative arterial paths, relatively high arterial pressure and almost zero pressure in the vein).

Burton-Opitz and Edwards (4), working with a stromuhr, obtained in dogs an average blood flow of 4.9 grams per gram of gland. This, as they point out, is very much more than the flow through any other organ with the possible exception of the thyroid. In connection with the figures given in the literature for the thyroid, it may be remarked that although there is no doubt that the blood flow through the normal thyroid is large in comparison with that of most organs, some of the experiments were probably performed on hyperplastic glands.

From the relatively great quantity of blood passing through the adrenals, it might be expected that the blood of the adrenal veins would differ less from arterial blood than ordinary venous blood does. It would hardly be credible, for instance, that the percentage loss of oxygen by the arterial blood in the adrenals should be as great as in the generality of tissues. It might, therefore, be looked for that adrenal vein blood should be richer in oxyhemoglobin than ordinary venous blood obtained, say, from the jugular vein.

If 5 grams of blood pass through a gram of adrenal in a minute, and the oxygen consumption of the gland is taken as 0.05 cc. (Neuman gives 0.045 cc.) per gram of organ per minute, the blood issuing from the adrenal veins would contain only 1 cc. of oxygen per 100 cc. of blood less than the arterial blood. In order that the difference should become as great as in ordinary mixed venous blood, say 8 volumes of oxygen per cent, the consumption of oxygen would have to rise to 0.4 cc. per gram of adrenal substance per minute. This would be five times as great as the oxygen consumption given by Barcroft for striated muscle during maximal activity. Yet a recent writer (5) thinks it worth while to demonstrate that the oxyhemoglobin bands in a dilution of adrenal vein blood are stronger than the oxyhemoglobin bands in jugular vein blood from the same animal, diluted to the same degree with oxygen-

free saline solutions, and to seek for an explanation of this fact in some action exerted by adrenalin in "augmenting the oxygen-absorbing capacity of hemoglobin."

The facts that adrenal vein blood often "develops an arterial appearance" in the vein when the latter is clamped at its outlet into the vena cava, and that on dilution with salt solution it becomes red sooner than ordinary venous blood are easily understood as soon as it is recognized that adrenal vein blood is nearer to arterial blood than ordinary venous blood. There is no evidence and no likelihood that adrenalin has anything to do with the matter at all.

"The observation that the addition of adrenalin to the perfusion fluid favorably affects the oxygen intake of the heart in perfusion experiments" ought not, I think, to suggest "that the base may play a similar rôle in relation to the oxygen-absorption of hemoglobin in the adrenal vein." For anything which strengthens the action of the heart may be expected to increase its oxygen consumption.

Again, it is known that the H-ion concentration of ordinary venous blood is somewhat greater than that of arterial blood. It might be expected from the great blood flow through the adrenal gland that the adrenal vein blood would be somewhat more alkaline than ordinary venous blood since it is nearer to arterial blood, and in particular cannot be supposed to have acquired as much carbon dioxide in its passage through the glands as mixed venous blood. The realization of this would probably have modified the statement in another paper (6)[1] that "the increased alkalinity (in the adrenal vein blood) is due to the dissolved adrenalin which it contains."

It is not easy to gather in what way these writers suppose that the change in the reaction of the adrenal vein blood is produced by the adrenalin in it. It is scarcely necessary to point out that it is highly improbable that the mere addition of the base adrenalin in the concentration of a $\frac{1}{500,000}$ molecular solution (which would correspond to a normal concentration of adrenalin in adrenal vein blood) to a liquid with the buffer properties of blood could produce an effect on the H-ion concentration measurable by the gas-chain method.

[1] The paper referred to, although headed "From the Cushing Laboratory for Experimental Medicine, Western Reserve University," was not seen by me until recently. The work was not done under my direction.

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